SYNTHESIS, CHEMICAL, SPECTRAL AND BIOLOGICAL STUDIES OF HETERO-STEROIDS

DISSERTATION

Submitted in Partial Fulfilment of the Requirements
for the Award of the Degree of

Master of Philosophy

IN

CHEMISTRY

BY

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C E R T I F I C A T E

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The dissertation entitled 'SYNTHESIS, CHEMICAL, SPECTRAL AND BIOLOGICAL STUDIES OF HETERO-STEROIDS', by Mr. Rao Uwais Ahmad Khan is suitable for submission for the degree of Master of Philosophy in Chemistry.

Shafiullah

(Prof. Shafiullah)

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Rao Uwais Ahmad Khan

(RAO UWAIS AHMAD KHAN)

TO MY PARENTS

C O N T E N T S

INTRODUCTION

Steroids, the derivatives of the perhydrocyclopentanophenanthrene nucleus, are widely distributed in nature and play an important role in the vital activities of living organisms. They have really, prodigious range of different biological and pnysiological activities which prompted the organic chemists to undertake the synthesis of heterosteroids of natural steroids in order to boost the all round activities. The physiological activities of steroidal hormones depend upon a number of factors including stereochemistry and overall shape of the molecule.

The pharmacological testings, along with the synthesis of new steroidal analogues have now become a major pre-occupation with organic chemists and continue to fascinate them the world over.

In recent years, it has become evident that by modification or introducing the hetero-ring in the naturally occurring steroids, it is possible to increase or decrease certain physiological properties. These observations are stimuting the expansion of steroidal chemistry with unusual carbon frame work and uncommon substituents at different positions in steroid nucleus.

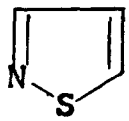
Our laboratory is mainly concerned with the synthesis of heterosteroids and their structure elucidation by chemical and spectral studies. An attempt has been made for the synthesis of halo derivative and its testing for the pharmacological activities along with the pharmacological testing of a steroidal thiazole.

C H A P T E R - O N E

THEORETICAL

SYNTHESIS OF THIAZOLE :

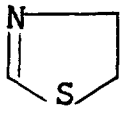
The five membered doubly unsaturated heterocyclic system containing one nitrogen and one sulfur atom is called thiazole but there are two possibilities for the arrangement of sulfur and nitrogen atoms in the ring : (I) Nitrogen and sulfur atoms are at 1,2-positions with the three carbon atoms, such an arrangement is known as isothiazole, (II) Nitrogen and sulfur atoms are arranged at 1,3-positions and called as thiazole.



(I)

(II)

The dihydrothiazoles (III) and (IV) are also called as thiazolines. Tetrahydrothiazole or thiazolidine (V) constitutes a well known and important class of compounds. Many polycyclic and fused ring systems containing the thiazole nucleus are also known.



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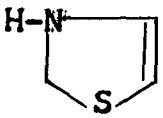

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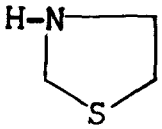


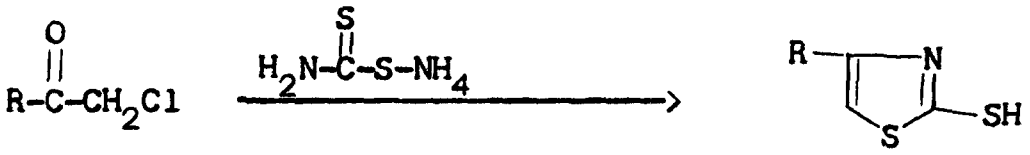
(V)

The history of true thiazole series began in 1879 with the work of Hofmann, who prepared derivatives of benzothiazole, such as 2-chlorobenzothiazole and 2-phenyl-benzothiazole[1]. Compounds containing the simple thiazole nucleus were first reported by Hantzch and co-workers[2].

After this work, knowledge of the thiazole system developed steading and many discoveries of commercial and biological interest gave impetus to the study. In 1888, Green described a yellow substance (Primuline base) and dihydrothio-p-toluidine that were obtained by fusion of p-toluidine with sulfur. These substances were immediately recognised as benzothiazole derivatives[3]. The investigations by Bogert et al.[4], greatly extended this field and reported in a series of papers begining in 1922. In 1922 Mill[5] recognised the value of cyanine dyes containing thiazole ring as photographic sensitizers. At about the same time 2-mercaptobenzothiazole was developed as rubber vuleaniza-tion accelerator and many related compounds were investi-gated[6]. Williams and et. al.[7] demonstrated the existence of the simple thiazole ring in vitamin B (Thiamine).

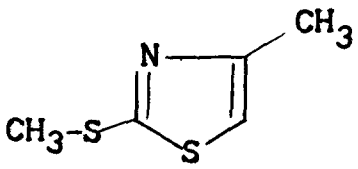
Miolati and Levi[8-10] reported the preparation of 4-methyl, 4-ethyl, 4-carbethoxy-methyl-, 4-phenyl-2-mercapto-thiazoles (VIIIa-d) by the condensation of the corresponding

halomethyl ketones (VIa-d) with ammonium dithiocarbamate (VII).



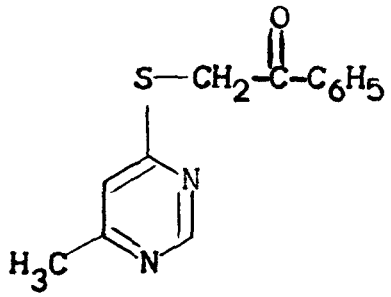
	R	(VII)		R
(VIa)	CH_3		(VIIIa)	CH_3
(VIb)	C_2H_5		(VIIIb)	C_2H_5
(VIc)	CH_2-$COOC_2H_5$		(VIIIc)	$CH_2COOC_2H_5$
(VId)	Ph		(VIIId)	Ph

2-Methylthio-4-methylthiazole (IX) was prepared by the reaction of chloroacetone (VIa) with dithiocarbamate[11,12].



(IX)

Singh et al.[13] reported that reaction of ω-(6-methyl-4-pyrimidinylthio)acetophenone (X) and 2-(6-methyl-4-pyrimidinylthio)cyclohexanone (XI) with aq. $HCl/HClO_4$ or $POCl_3$ followed by hydrolysis provided 1-(4-aryl-2-thiazolyl)-2-propanone (XII) and 4,5,6,7-tetrahydro-2-acetonyl benzothiazole (XIII), respectively.



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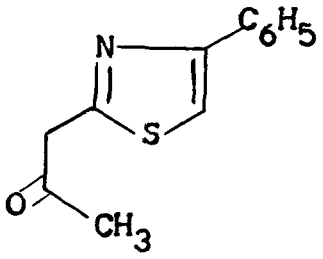

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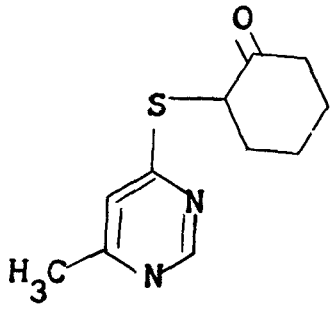

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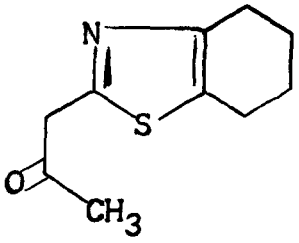
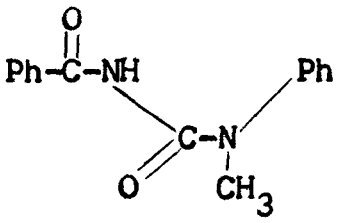


(XIII)

Meakins et al.[14] reported that the reaction between N-benzoyl-N'-methyl-N'-phenylthiourea (XIV) and chloroacetone (VIa) gave 5-benzoyl-4-methyl-2-(N-methyl-N-phenyl amino)thiazole (XV).



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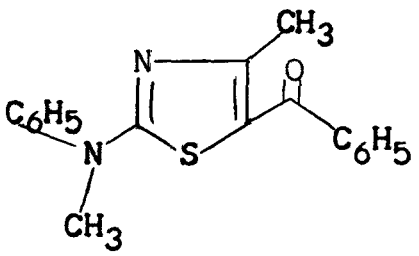
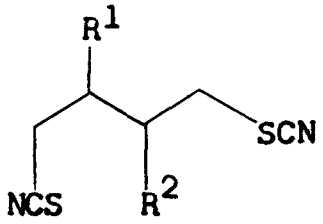
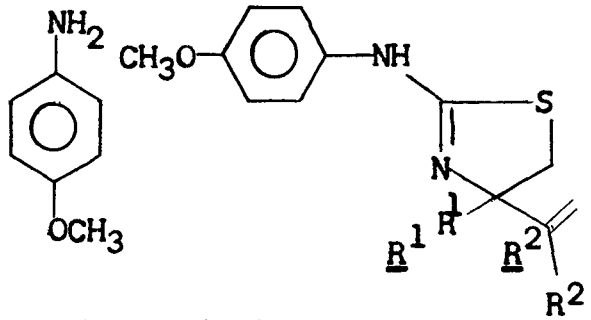


(XV)

The reactions of dithiocyanates (XVI-XVIII) with p-methoxyaniline (XIV) in hot benzene afforded the dihydrothiazoles (XX-XXII)[15].

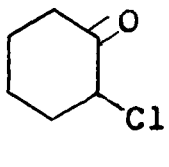


	R^1	R^2
(XVI)	H	H
(XVII)	CH_3	H
(XVIII)	CH_3	CH_3



	R^1	R^2
(XX)	H	H
(XXI)	CH_3	H
(XXII)	CH_3	CH_3

Condensation of 2-chlorocyclohexanone (XXIII) with substituted thioamide (XXIV-XXX) in hot ethanol gave 2-substituted 4,5,6,7-tetrahydrobenzothiazole derivatives (XXXI-XXXVII)[16].



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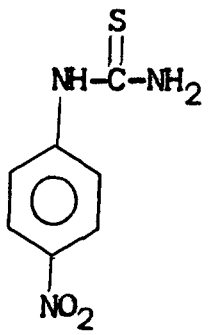

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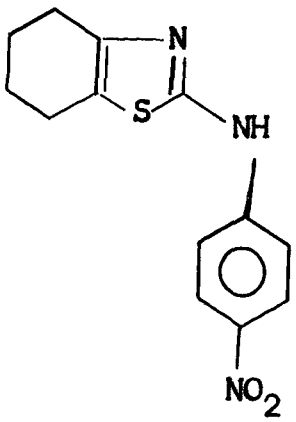
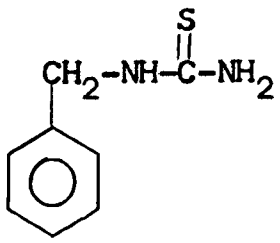


(XXXI)



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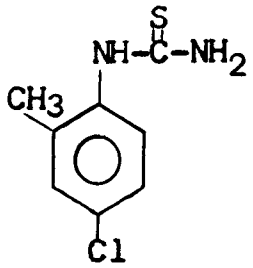

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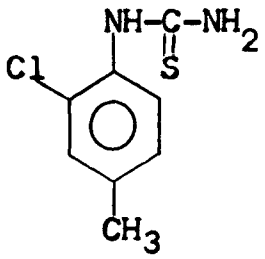

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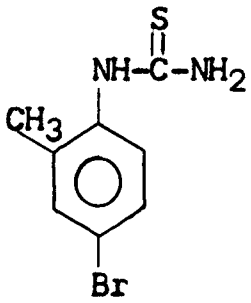

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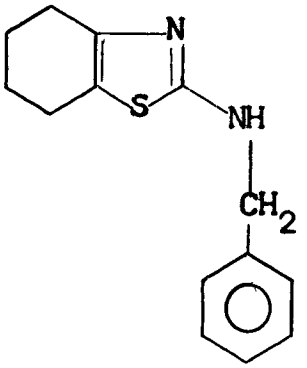

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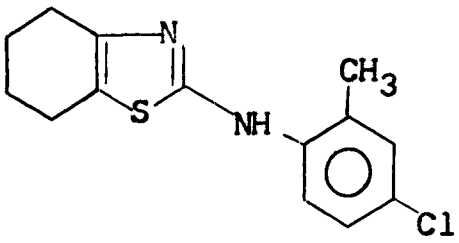

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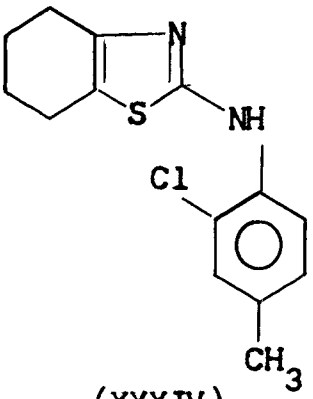

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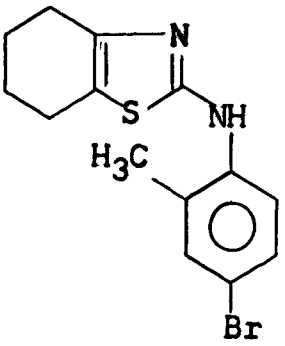

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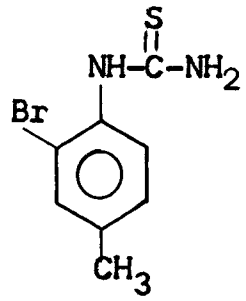

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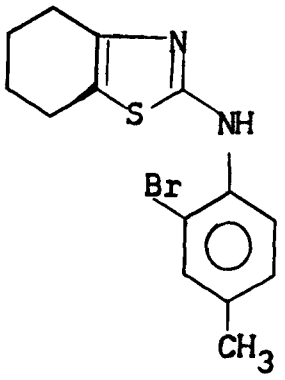

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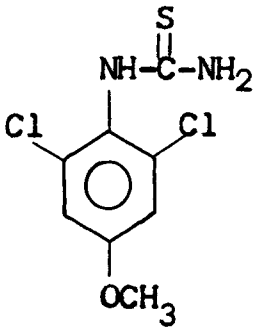

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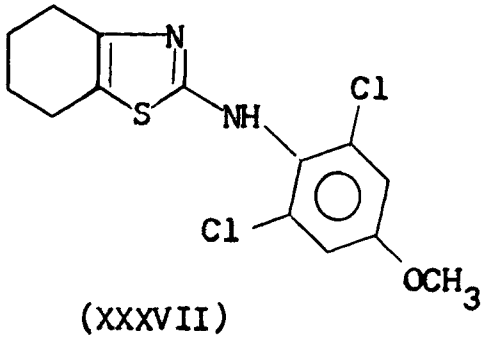
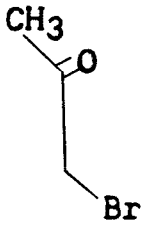
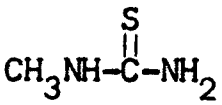


(XXXVII)

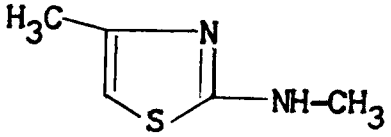
The condensation of α-bromoketone (XXXVIII) with N-methyl thiourea (XXXIX) in neutral solvent afforded exclusively 2-N-methylamino-(4-substituted)thiazoles (XL)[17].



(XXXVII)



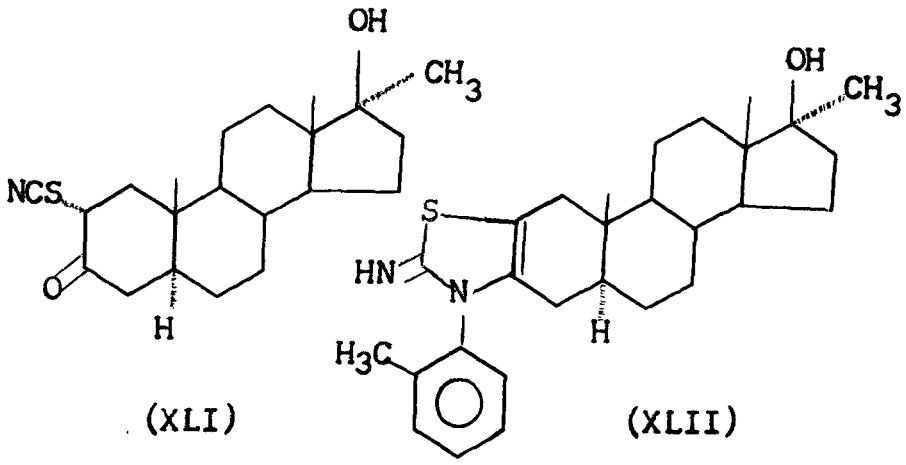
(XXXIX)



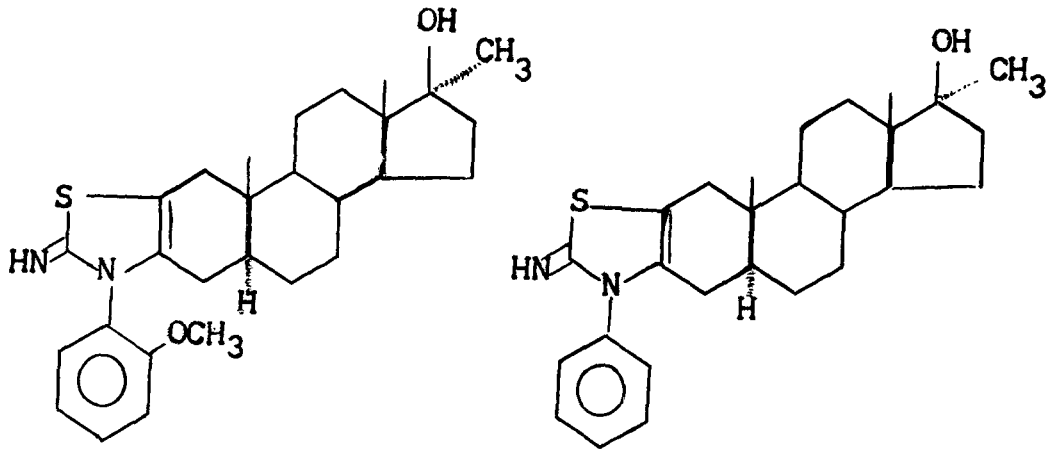
(XL)

The reaction of 2-amino-4-chlorothiophenol with various aldehydes and ketones have been studied extensively[18,19].

During the last few years, a large number of steroidal thiazoles have been synthesised by different routes. When a mixture of O-toluidine-hydrochloride and 2α-thiocyanato-17α-methyl-androstan-3-en-17β-ol (XLI) in ethanol was refluxed with ethyl acetate provided 17α-methyl-17β-hydroxyandrostano [3,2-d]-2',3'-disubstituted thiazolines (XLII-XLIV)[20].

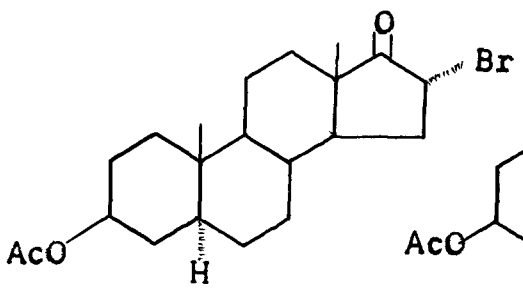


(XLI) (XLII)



(XLIII) (XLIV)

When 3β-acetoxy-5α-androstan-(6α-bromo-17-one) (XLV) was treated with thiourea provided 3β-acetoxy-5α-androstano [17,16-d]-2'-aminothiazole (XLVI)[21].



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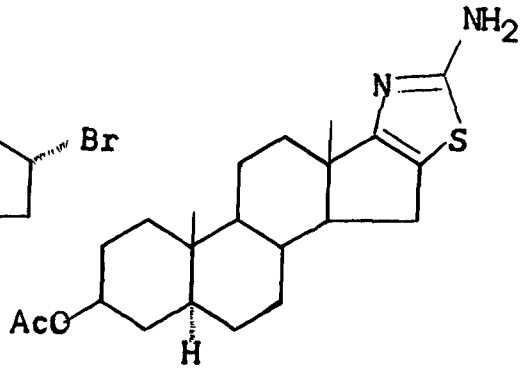
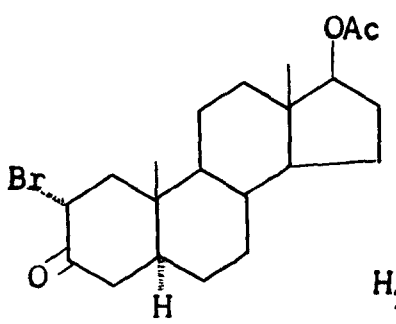
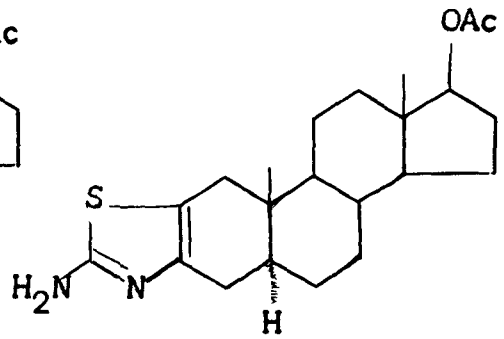


(XLVI)

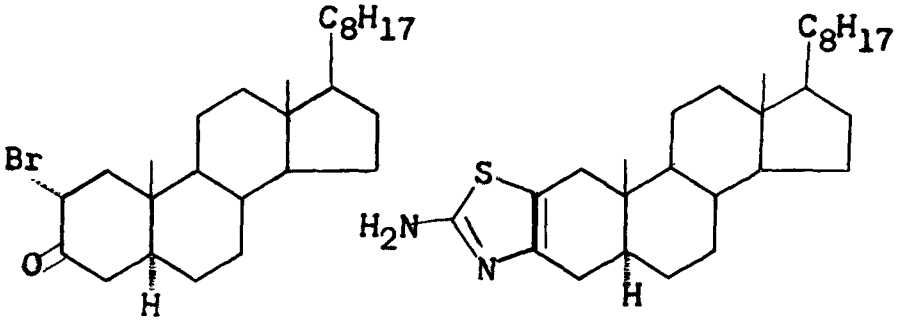
Similar treatment of 17β-acetoxy-2α-bromo-5α-androstan-3-one (XLVII) and 2α-bromo-5α-cholestan-3-one (XLVIII) with thiourea in isopropyl alcohol provided 17β-acetoxyl-5α-androstano[3,2-d]-2'-aminothiazole (XLIX) and 5α-cholestano[3,2-d]2'-aminothiazole (L)[22].



(XLVII)



(XLIX)

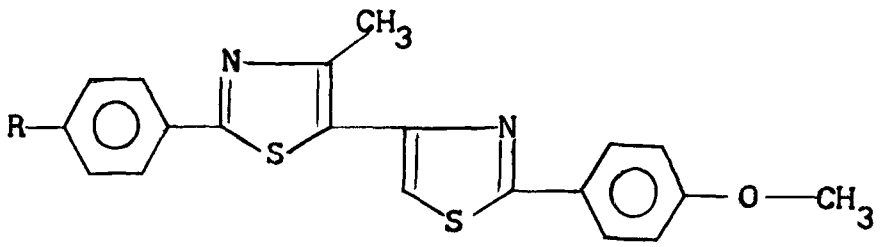


(XLVIII) (L)

The Pharmacological Studies of Steroidal Thiazole :

The substituted thiazoles have been reported to possess many important pharmacological actions which prompted us for the studies. Irismetov et. al.[22] have reported certain thiazolosolasodanes possessing antimicrobial activity. Some guanidinothiazoles synthesised, in the Imperical Chemical Industries Ltd.[23], possess antihistaminic actions. Tartler and Weuffen[24] reported fungistic properties of some thiazole and thiazole derivatives.

Joshi et. al.[25] synthesised some fluorine containing 2-(N-aryl amino/or -methyl-4-aryl)thiazoles showing the antibacterial activity. Sawney and Arora[26] have studied the antiinflammatory activity of some 2'-Alkyl/Aryl-2-aryl-4-methyl-4',5'-bithiazolyls and 2'-amino substituted amino-2-aryl-4-methyl-4',5-bithiazolyls by carrageenin oedema test in rats.



(I) R = H, (LII) R = Cl (LIII) R = O-CH_3

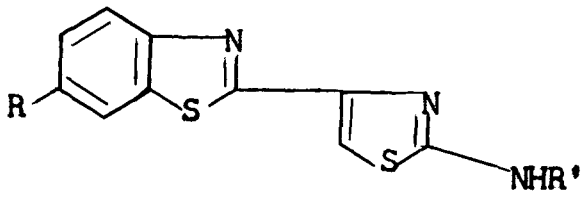
[LI, LII and LIII - Bithiazolyls]

They found the compound to inhibit the oedema by 16 to 41% at the dose of 100 mg/kg. The highest activity was found in the compounds possessing anisyl groupings with thiazole moieties. The LD_{50} for the compound was 800 mg/kg or more. The compounds were also treated in vitro for anti-tubercular activity against M. tuberculosis but were found to be inactive.

Sawney et. al.[27] synthesized as well as tested for antiinflammatory activity of some 2-(2'-amino-4-thiazolyl)-benzothiazoles also. The activity was measured in rat by 'Carrageenin in foot oedema test.' Groups of 5 adult rats were used for each dose of 100 mg/kg of the test compounds which were administered interaperitoneally 1 hour before injecting Carrageenin and the volume of the foot was measured

3 hours after injection. The results were given in term of percentage (%) with respect to the controlled. The percentage inhibition for the different drugs is as :

(iv) 60%, (v) 56%, (vi) 41%, (vii) 61%, (viii) 58%.

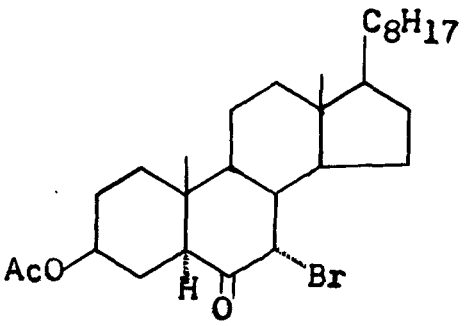


(LIV) R=H, R'=H (LV) R=H, R'=Ph (LVI) R=H, R'=p-ClC_6H_4

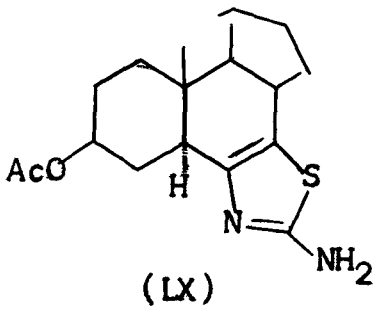
(LVII) R=H, R'=O-$CH_3C_6H_4$ (LVIII) R=H, R'=p-CH_3-O-C_6H_4

Steroidal Thiazole and its Biological Activities :

The non-steroidal substituted thiazoles have been reported to possess biological activities[22-27]. These reported observations prompted to synthesise the steroidal thiazole (LX) from easily accessible steroidal α-bromo-ketone (LIX) with the reaction of thiourea.



(LIX)



(LX)

Reaction of 3β-acetoxy-7α-bromo-5α-cholest-6-one with thiourea :

3β-Acetoxy-7α-bromo-5α-cholest-6-one (LIX) was refluxed in ethanol with thiourea for a period of 8 hours. The mixture was diluted with water and extracted with ether. The ethereal layer was washed with water and dried over anhydrous sodium sulfate and crystallised which provided a compound having m.p. 202°C.

Characterisation of the compound, m.p. 202°C as 3β-acetoxy-2'-amino-5α-cholest-6-ano[6,7-d]thiazole (LX) :

The compound (LX) was analysed correctly for $C_{30}H_{48}N_2O_2S$. The IR spectrum of the compound showed weak absorption bands at 3450, 3300, 3150 cm^{-1} and strong absorption bands at 1730, 1265 cm^{-1} which are due to presence of NH_2 and acetate groups. Other bands were observed at 1630 (C=C), 1525 (C=N), 1460, 1375 (C-N) and 665 (C-S). These values indicate the presence of thiazolyl moiety[28] in the steroid nucleus. The ^{1}H-NMR spectrum of the compound displayed a broad singlet at δ 5.3 integrating for two protons assigned to $-N\underline{H}_2$ (exchangeable with deuterium) and other broad multiplet centred at δ 4.71 for C3α-$\underline{H}$ (W1/2 = 18Hz)[29]. The signal at δ 2.05 integrating for three protons was due to acetyl methyl. Angular and side chain methyl protons were observed at δ 1.2 (C_{10}-CH_3), 0.78 (C_{13}-CH_3) and 0.91, 0.81. On the basis of these analytical and spectral data, the compound, m.p. 202°C was characterized as 3β-acetoxy-2'-amino-5α-cholest-6-ano[6,7-d]thiazole (LX)[30].

A new thiazole, 3β-acetoxy-2'-amino-5α-cholest-6-ano[6,7-d]thiazole was subjected to the brief preliminary screening at the various doses for the biological activity.

The substituted thiazoles have not yet been reported to possess central actions even though the compound was tested for neuropharmacological actions, as it is usual to begin with 'Blind Screening in this manner'. As the other thiazoles[24-27] have been shown to possess antiinflammatory action, therefore, the test compound was studied for that action also.

The observation that the test compound reduced the frequency, co-ordination of movements and palpebral aperture indicates that it possessed central depressant action. The depression of the rate of the respiration and decrease in the body temperature would be due to specific central actions or be a part of generalised central depression. The reduction in the tone of the limbs and abdomen could be due to effect on motor centres and tracts or due to generalised central depression. The increase in reaction time to thermal pain producing stimulus indicates the presence of analgesic action probably of the opioid type. The observation of Straub's response in the animals treated with the highest dose supports this inference. The prolongation of the reaction time to mechanical nociceptive stimulus by the highest dose indicates anticonvulsant action[31]. The absence of touch response in the animals treated by highest dose could be due to interference in the

sensory path way but it is more likely that reflects a greater central depression. The near absence of spontaneous locomotion as well as almost instaneous effect observed with the highest dose indicates that it produces more intense central depression and supression of pain produced mechanical stimules indicates that it also has some additional central action as compared to the lower doses. However, the 20% mortality produced by the dose reflects its toxicity. The absence of any effect on the pinna and corneal reflux shows that the test compound does not block the reflex path way. The fact that the test compound did not produce exophthalmo usand did not increase laerimation, salivation and urination indicates that it lacks effect on Autonomics Nervous System. The thiazole produced highly significant inhibition of carrageenin induced oedema, thus it is seen to possess antiinflammatory activity.

The present study therefore reveals that the test compound i.e. 3β-acetoxy-2'-amino-5α-cholest-6-ano(6,7-d) thiazole possesses central depressant action and analgesic action at the dose of 5 mg/kg body weight. It may also possess central muscle relaxant action and specific respiratory depressant and hyperthermic action. The analgesia could probably be of the opioid type. The highest dose i.e.

30 mg/kg possesses more intense central actions and probably additional central action such as anticonvulsant action. The study also demonstrates that the compound possesses highly significant antiinflammatory activity. The later effect has been seen in other thiazoles but the nuemerous neuopharmacological actions have been shown for the first time in this compound.

'The neuropharmacological and antiinflammatory action of a new thiazole compound'.

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29th Annual Convention of Chemists, MED-22, H-10 (1992).

EXPERIMENTAL

EXEPRIMENTAL :

All melting points were observed on a Kofler hot block apparatus and are uncorrected. IR spectra were obtained in Nujol with a Pye-Unicam SP3-100 Spectrophotometer. IR values are given in cm^{-1}. ^1H-NMR spectra were run in $CDCl_3$ on a varian A-60D instrument with Me_4Si as internal standard. Values are given in ppm (δ). T.L.C. plates were coated with silica gel and sprayed with 20% aqueous solution of perchloric acid.

3β-Acetoxycholest-5-ene (LXI) :

A mixture of cholesterol (50 g), pyridine (75 ml, freshly distilled over KOH) and acetic anhydride (50 ml) was heated on a water bath for 2 hours. The resulting brown solution was poured on to crushed ice-water mixture with stirring. A white solid thus obtained was filtered under suction and washed with water so as to remove pyridine and air dried. The crude product on recrystallisation from acetone gave pure 3β-acetoxy cholest-5-ene (47.0 g), m.p. 115°C (reported[32], m.p. 116°C).

3β-Acetoxy-6-nitrocholest-5-ene (LXII) :

3β-Acetoxycholest-5-ene (10 g) was covered with nitric acid (250 ml; d 1.52) and sodium nitrite (10 g) was gradually added over a period of 1 hour with continuous stirring. Slight cooling was also maintained during the course of reaction. Stirring was continued for 2 hours more. When the yellow spongy mass was separated on the surface of the mixture, it was diluted with cold water. The whole mass was extracted with ether and washed with water and sodium bicarbonate (5%) and water. The ethereal layer was then dried over anhydrous sodium sulfate. Removal of the solvent provided oil which was crystallised from methanol to yield (LXII) (6.8 g), m.p. 103° (reported[33], m.p. 102-104°C).

3β-Acetoxy-5α-cholestan-6-one (LXIII) :

3β-Acetoxy-6-nitrocholest-5-ene (6.0 g) was dissolved in acetic acid (250 ml) and zinc dust (12 g) was added in small portions with shaking. The suspension was heated under reflux for 4 hours, water (12 ml) was added during the reaction. The hot solution was filtered, cooled to room temperature and diluted with water. The precipitate obtained was taken in ether and ethereal layer was washed with water, sodium bicarbonate solution (5%) and water and dried over

anhydrous sodium sulfate. Removal of the solvent gave an oil which was crystallised from methanol to give the ketone (LXIII) (3.9 g), m.p. 128° (reported[34], m.p. 127-128°C).

α-Bromination of 3β-acetoxy-5α-cholestan-6-one (LXIII) : 3β-Acetoxy-7α-bromo-5α-cholest-6-one (LIX) :

A solution of bromine (2.12 g Br_2 in 25 ml acetic acid) was added dropwise to a solution of 3β-acetoxy-5α-cholestan-6-one (LXIII) (6.0 g, in 75 ml ether and 15 ml acetic acid) and was refluxed for 22 hours. After usual work up and evaporation of the ether gave an oil which on crystallisation from methanol provided bromoketone (LIX) (5.2 g), m.p. 146° (reported[35], m.p. 145°C).

Reaction of 3β-acetoxy-7α-bromo-5α-cholestan-6-one (LIX) with thiourea : 3β-Acetoxy-2'-amino-5α-cholest-6-ano [6,7-d]thiazole (LX) :

3β-Acetoxy-7α-bromo-5α-cholestan-6-one (LIX) (1.25 g) in ethanol (25 ml) was refluxed with thiourea (0.181 g) for 8 hours. The reaction mixture was diluted with water and extracted with ether. The ethereal layer was washed with water and dried over anhydrous sodium sulfate. Evaporation

of the solvent gave an oil which was crystallised from petroleum benzene to provide the thiazole (LX), 0.875 g, m.p. 201°C (reported[30], m.p. 202°C).

Analysis found : C, 71.8; H, 9.5; N, 5.6

Required : C, 72.0; H, 9.6; N, 5.6%

I.R. : $\nu_{max.}$ 3450, 3300, 3150 (NH_2), 1730, 1265 (CH_3COO-) and 1630 (C=C), 1525 (C=N), 1460, 1375 (C-N) and 665 cm^{-1} (C-S).

^{1}H-NMR : ∂ 5.3 (brs, $N\underline{H}_2$, exchangeable with deuterium), 4.71 (brm, W1/2 = 18Hz; C3α-$\underline{H}$), 2.05 (s, $C\underline{H}_3COO$-), 1.20 (C_{10}-$C\underline{H}_3$), 0.78 (C_{13}-$C\underline{H}_3$), 0.91 and 0.81 (other methyl protons).

PHARMACOLOGICAL TESTING :

Gross behaviour test of 3β-acetoxy-2'-amino-5α-cholest-6-ano[6,7-d]thiazole :

It was carried out in mice by the method of Smith[36] modified by us in the light of Irwin's method[37]. This is a simple and comprehensive test detecting a large number of neuropharmacological actions. As the test compound lacks pharmacological history, therefore, the first dose used was 30 mg/kg body weight of animals. However, lower doses were

used subsequently as the above dose produced some mortality. The compound was dissolved in propylene glycol and administered intraperitoneally. The effect of the steroidal thiazole was studied at the dose of 5 mg/kg, 10 mg/kg, 20 mg/kg, 25 mg/kg and 30 mg/kg. Each dose was tested in 6 mice of either sex weighing 20-25 gms. The animals were observed for various parameters listed in Table-I. The mechanical and thermal nociceptive stimulus was given by applying a sheathed bull dog clamp at the tail and by placing the animal on Eddy's Hot Plate at 55.5^{o}C respectively. A group of 6-untreated mice was used for 6 hours and then at 24 hours. They were observed subsequently for mortality at 24 hours for 6 days.

In the gross behaviour test, it was observed that all the doses of the test compound reduced the spontaneous locomotion and made the movements uncoordinated. The palpebral aperture was reduced. The rate of respiration was decreased and the depth increased. The body temperature, was observed by palpation, was reduced and piloerection was produced. The limb and abdominal tone was reduced but pinna reflex and corneal reflex were uneffected. Lacrimation, Salivation and excessive urination were not observed. The defecation was normal. The reaction time to the thermal and mechanical pain producing stimulii was increased.

Straub's response was observed in half of the animals administered with the highest dose. One of the animal was found to be dead on the 3rd day. The effect of highest dose developed instaneously, while the rest of the doses produced the effect 15 minutes after drug administration. The finding are presented in Table-I.

TABLE-I Effect of various doses of steroidal thiazole (LX) on behaviour of mice

S. No.	Parameters	3β-Acetoxy-2'-amino-5α-cholest-6-ano[6,7-d thiazole				
		5 mg/kg	10 mg/kg	20 mg/kg	25 mg/kg	30 mg/kg
1.	Time of first unusual S/S	15 mints	15 mints	15 mints	15 mints	0 mints
2.	Movements	↓	↓	↓	↓	↓↓
3.	Coordination	↓	↓	↓	↓	↓
4.	Palpebral-aperture	↓	↓	↓	↓	↓
5.	Alertness	↓	↓	↓	↓	↓
6.	Grooming	N	N	N	N	N
7.	Restlessness	A	A	A	A	A
8.	Stereotypy	A	A	A	A	A
9.	Tremor	A	A	A	A	A
10.	Convulsions	A	A	A	A	A
11.	Rate of respiration	↓	↓	↓	↓	↓
12.	Depth of respiration	↑	↑	↑	↑	↑

TABLE-I Contd....

13.	Body temperature	↓	↓	↓	↓	↓
14.	Piloerection	+Ve	+Ve	+ve	+ve	+ve
15.	Limbtone	↓	↓	↓	↓	↓
16.	Abdominal tone	↓	↓	↓	↓	↓
17.	Pinna reflex	N	N	N	N	N
18.	Corneal reflex	N	N	N	N	N
19.	Lacrimation	A	A	A	A	A
20.	Salivation	A	A	A	A	A
21.	Urination	N	N	N	N	N
22.	Excessive defecation	No	No	No	No	No
23.	Startle response	+ve	+ve	+ve	+ve	+ve
24.	Touch response	+ve	+ve	+ve	+ve	+ve
25.	Straub's response	-ve	-ve	-ve	-ve	+ve
26.	Mortality	Nil	Nil	Nil	Nil	1/6

N = Normal, A = Absent, +ve = Positive, -ve = Negative

Carrageenin Induced Oedema Test of 3β-acetoxy-2'-amino-5α-cholest-6-ano[6,7-d]thiazole (LX) :

It was carried out in albino rats of either sex weighing 150-200 gms by the method of Winter et. al.[38] Twelve rats were devided into 2 groups of 6 animals each. The animals in

the test group were administered with 17.5 mg/kg of the test compound dissolved in propylene glycol (injected intraperitoneally) while the control animals were administered with equal volume of propylene glycol in the same manner. One hour later 0.05 ml of 1% solution of carrageenin in normal saline was injected subcutaneously under the plantar aponeurosis of the right paw of all the animals. The thickness of the paw was measured by an electronic micrometer before the injection of the carrageenin and 3 hours after it. The mean increase in thickness of the control and test groups was compared by student's 't' test and the percentage inhibition of the thickness in the animals was also calculated.

In the carrageenin induced oedema test, it was found that the mean increase in thickness of the right hind paw was 2.34 $\pm$ 0.54 mm in the control animals while in the test animals it was found to be 0.86 $\pm$ 0.09 mm. Thus increase in the hind paw thickness was greatly reduced in the test animals ($p < 0.01$). The percentage inhibition of thickness in the test animals was to be 66.6%. The results are summarised in Table-II.

TABLE-II. Effect of 3β-acetoxy-2'-amino-5α-cholest-6-ano[6,7-d]thiazole on Carrageenin Induced Oedema in Rat Paw :

Drug	Thickness of Rat's Paw in mm (mean ± S.E.)		Increase in Thickness of Paw in mm (mean ± S.E.)	% Inhibition of Inflammation	'P' Value
	Before Carrageenin injection	3 hours after C. injection			
Control (3.33 ml/kg Propylene glycol)	2.997 ± 0.071	5.499 ± 0.352	2.34 ± 0.54		
Test Compound in Propylene glycol (17.5 mg/kg)	2.921 ± 0.129	3.76 ± 0.098	0.86 ± 0.09	66.6	< 0.01

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C H A P T E R - T W O

THEORETICAL

SYNTHESIS OF STEROIDAL KETONES AND THEIR BROMINATION

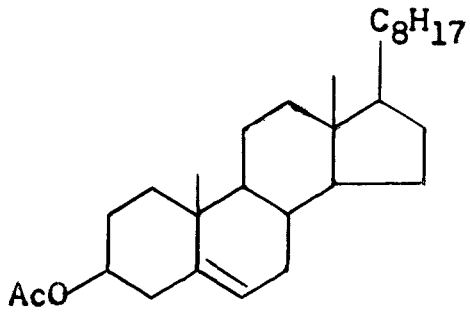
The development of new reaction pathways in the synthetic organic chemistry by oxidation methods[1] have been very much useful for the organic chemists. Succeptibility of alkenes and hydroxy group to the electrophilic attack and their capability to reduce most of the oxidants motivated the chemists to study the oxidising effects of transition metal ions and oxides on alkenes and hydroxy group. The importance of metal ion as oxidants is due to their low cost, stability and considerably selectivity with respect to functional groups by virtue of their action.

The most commonly used inorganic oxidants are Potassium permanganate, Osmium tetraoxide, Ruthenium tetraoxide, Palladium chloride, Potassium peroxydisulphate and certain chromium and mercury salts. Besides these, some organic complexes of these metals are also used for example Pyridinium dichromate, Butyl chromate etc.

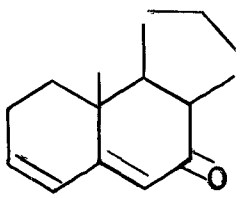
The oxidation of alcohols mainly give carbonyl compounds. The primary alcohols give rise to the aldehydes and secondary alcohols yield ketones. While alkenes undergo either exhaustive oxidation or allylic oxidation depending upon the reaction conditions, reagents used and

also upon the nature of oxidants. In some cases, the reactions take place at room temperature but in most of the cases, the reactions are carried out at elevated temperatures and in some of the cases the reactions even take place at low temperatures to avoid the extensive oxidation. The products of these oxidations from olefins are ketones, α-ketols and other degraded products.

Windus and Naggatz[2] reported the oxidation of cholesteryl acetate (I) with chromic acid in acetic acid which yielded cholesta-3,5-dien-7-one (II).

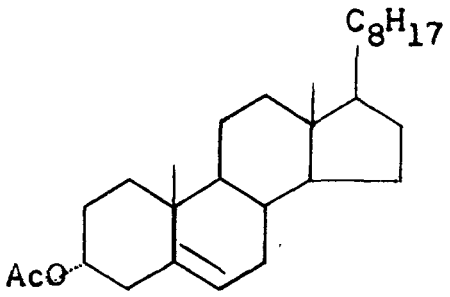


(I)

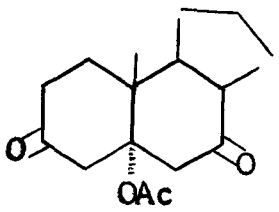


(II)

Fieser et. al.[3,4] identified the oxidation product of Windus and Naggatz obtained by chromic acid oxidation from epicholesteryl acetate (III) as 5-acetoxy-5α-cholestan-3,7-dione (IV) and suggested the intramolecular C_3-C_5 acyl migration.

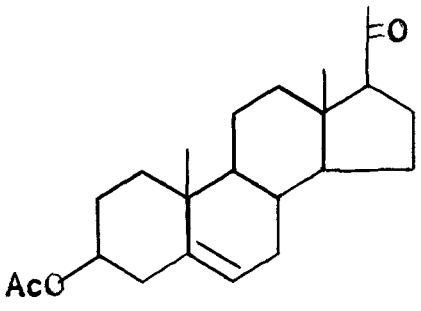


(III)

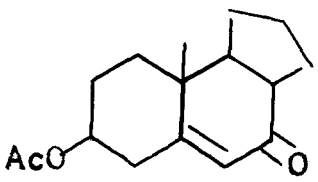


(IV)

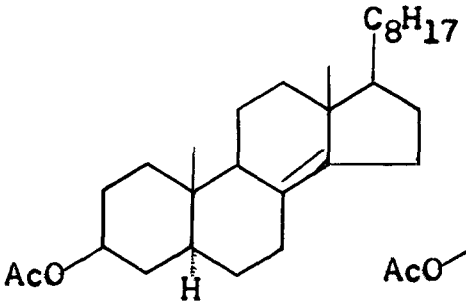
The formation of α,β-unsaturated ketones has also been reported from the oxidation of steroidal alkenes with chromic acid. Marshall et. al.[5] reported the introduction of ketone (VI) at C-7 of pregnolone acetate (V) using sodium chromate in acetic acid - acetic anhydride solution. (Δ^{8-14}) cholesteryl acetate (VII) with chromium trioxide and acetic acid yielded five products[6] (VIII-XII). Cholesteryl acetate (I) with chromic acid gave 5,6-seco product[7] (XIII).



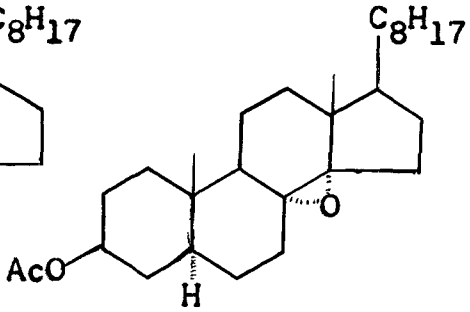
(V)



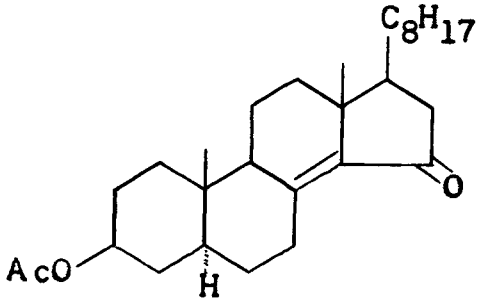
(VI)



(VII)



(VIII)



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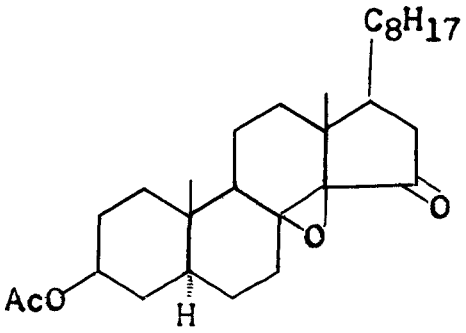
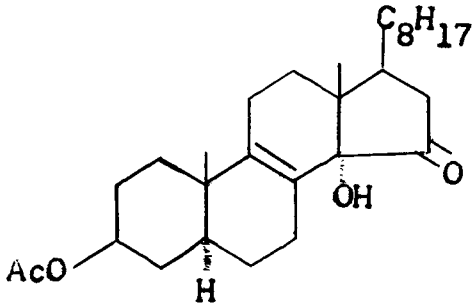
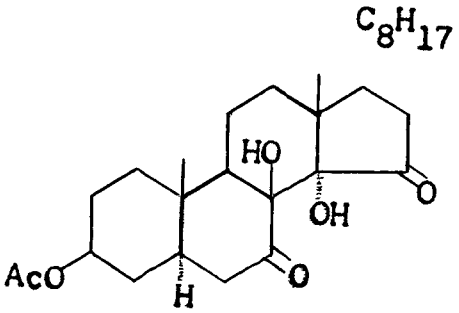


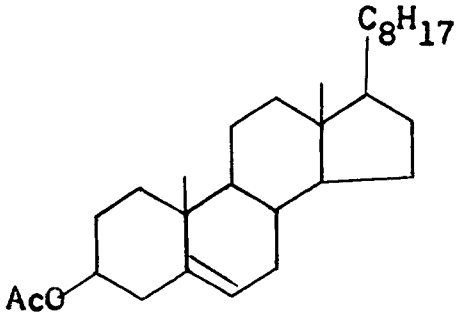
(X)



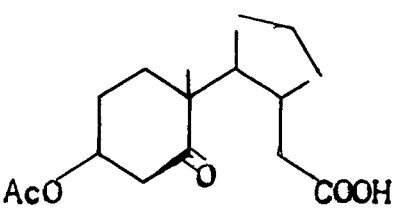
(XI)



(XII)

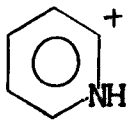


(I)



(XIII)

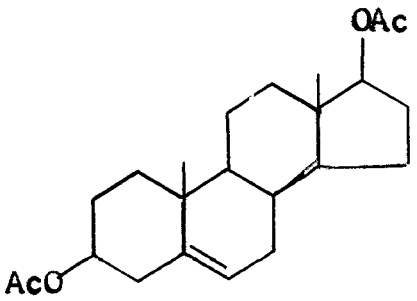
Corey and Suggs[8] have studied the use of Pyridinium chlorochromate as oxidant. They reported the advantage of this reagent over others and showed that it can be prepared easily, safely and also possesses high capability to convert primary alcohols into aldehydes in better yield.

$$CrO_3 + HCl \rightleftharpoons HCrO_3Cl \xrightarrow{Py}$$  $$[CrO_3Cl]^-$$

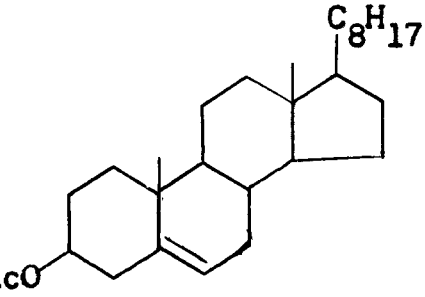
$$R - \underset{\underset{OH}{|}}{CH} - R' +$$  $$[CrO_3Cl]^- \longrightarrow R - \underset{\underset{O}{\|}}{C} - R'$$

William and co-workers[9] reported the oxidation of olefins (I, XIV-XXVI) with chromium trioxide-pyridine complex at room temperature and obtained the following products (XXVII-XLV) in good to moderate yield.

Olefin

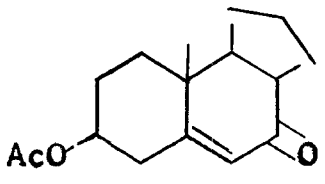


(XIV)

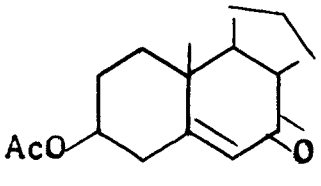


(I)

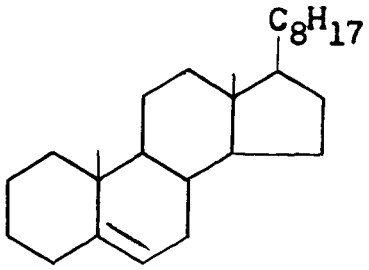
Ketone



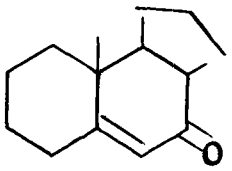
(XXVII)



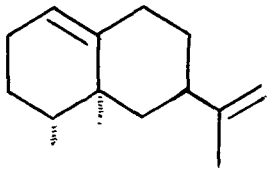
(XXVIII)



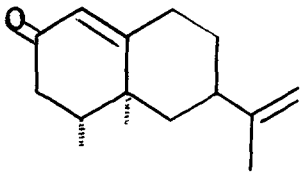
(XV)



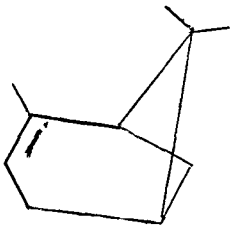
(XXIX)



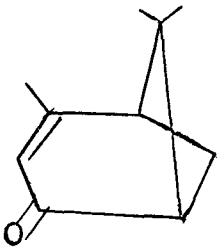
(XVI)



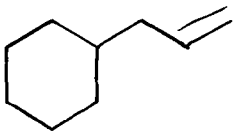
(XXX)



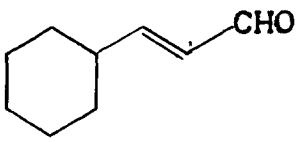
(XVII)



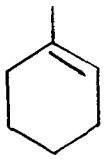
(XXXI)



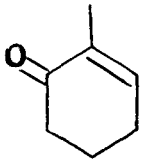
(XVIII)



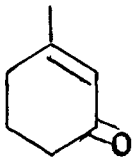
(XXXII)



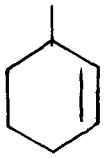
(XIX)



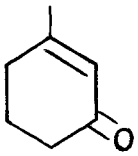
(XXXIII)



(XXXIV)

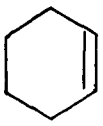


(XX)

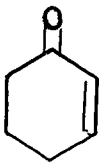


(XXXV)

(XXXVI)

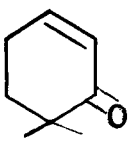


(XXI)

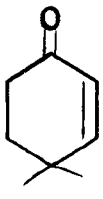


(XXXVII)

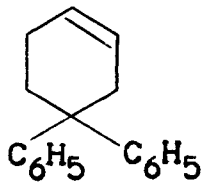
(XXII)



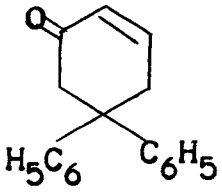
(XXXVIII)



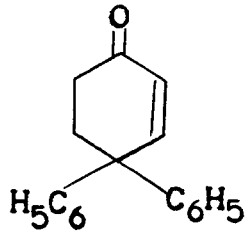
(XXXIX)



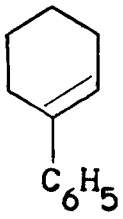
(XXIII)



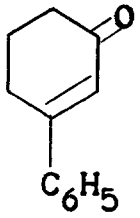
(XL)



(XLI)



(XXIV)



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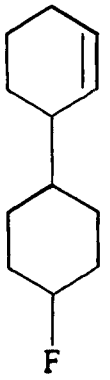

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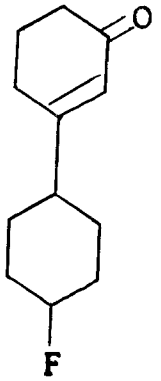
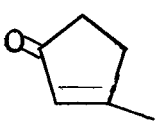
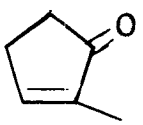


(XLIII)

(XXVI)

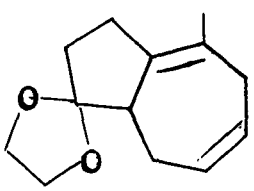


(XLIV)

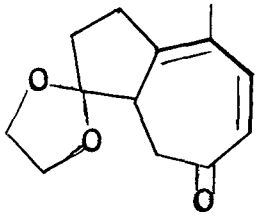


(XLV)

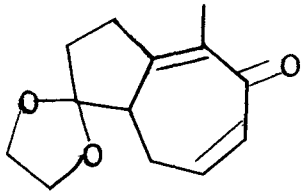
Wender et. al.[10] reported the oxidation of 1,4-diene (XLVI) by Pyridinium chlorochromate and obtained dienones (XLVII) and (XLVIII). Marshall and Wuts[11] reported on analogous behaviour of 1,4-diene (XLIX) with Pyridinium chlorochromate which yielded two products (L and LI).



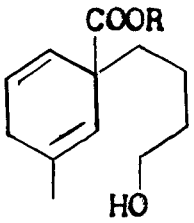
(XLVI)



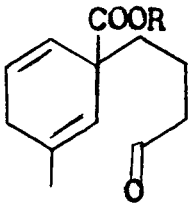
(XLVII)



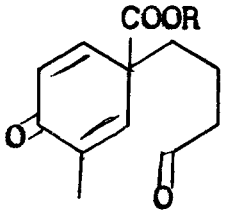
(XLVIII)



(XLIX)

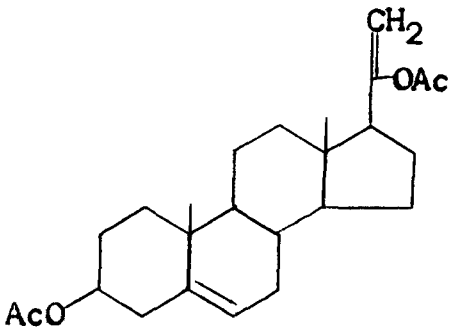


(L)

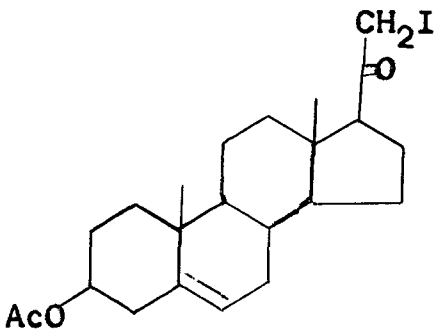


(LI)

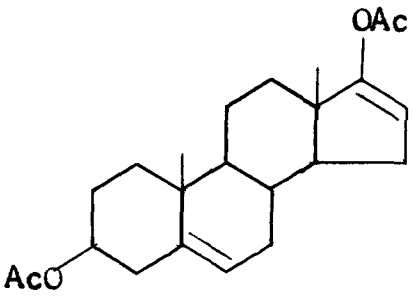
Djerassi and Lenk[12] reported the use of N-iodosuccinimide for the synthesis of iodoketones. They treated steroidal enol acetate (LII) with N-iodosuccinimide and obtained 21-iodopregnenolone acetate (LIII). Muller and Jones[13] prepared, α-iodoketone (LV) from the enol acetate (LV).



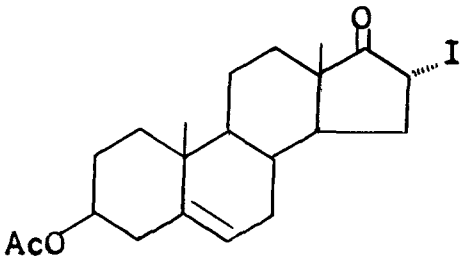
(LII)



(LIII)

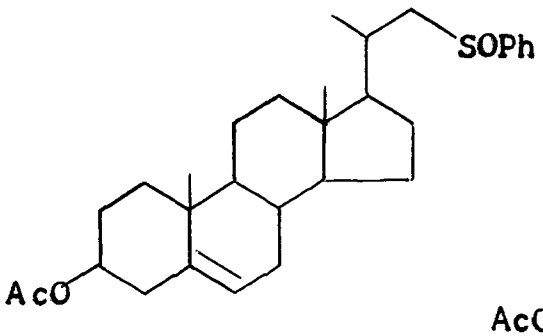


(LIV)

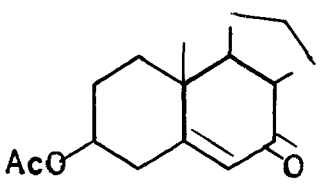


(LV)

Siemann et. al.[14] reported the preparation of 3β-substituted (20S)-20-[benzenesulphonyl)methyl]pregn-5-en-7-one (LVII) from respective 3β-substituted (20S)-20-[(benzenesulphonyl)methyl]pregen-5-ene (LVI).

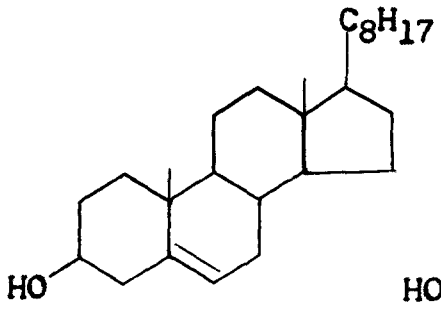


(LVI)

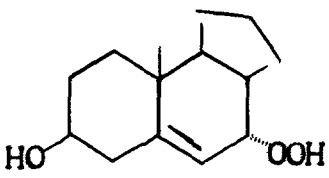


(LVII)

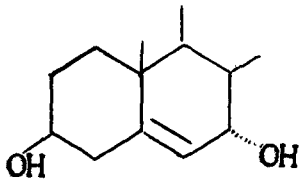
Cholesterol (LVIII) ubiquitously present in mammalian tissues is subjected to autoxidation by air, peroxidation <u>in vivo</u> and metabolism. These intriguing events and biologically active oxysterols (LIX - LXXIV) thus produced, were reviewed in detail[15-17].



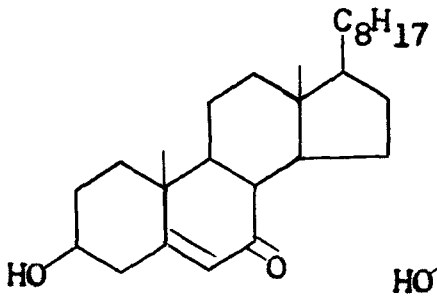
(LVIII)



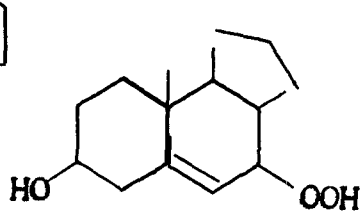
(LIX)



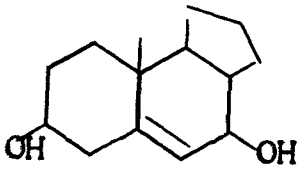
(LX)



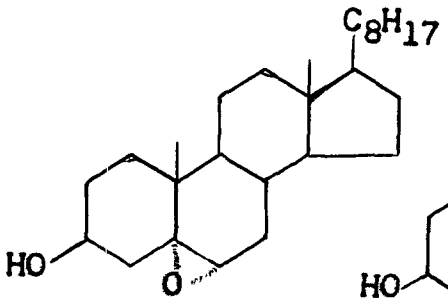
(LXI)



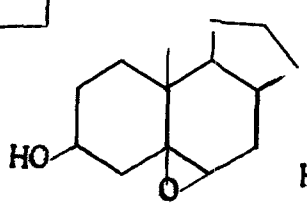
(LXII)



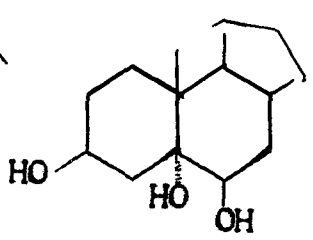
(LXIII)



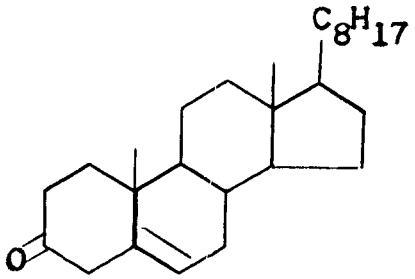
(LXIV)



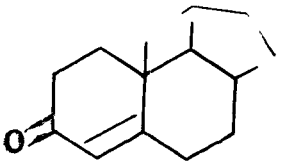
(LXV)



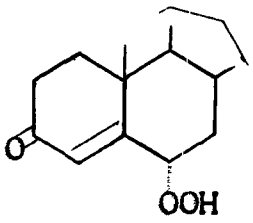
(LXVI)



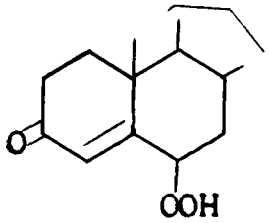
(LXVII)



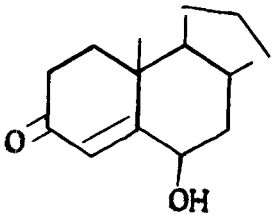
(LXVIII)



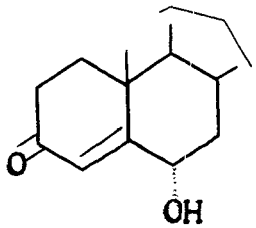
(LXIX)



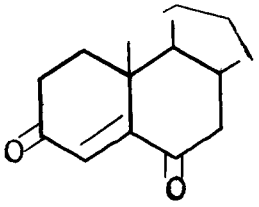
(LXX)



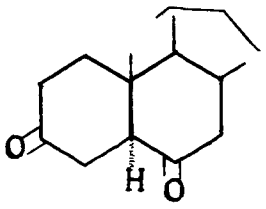
(LXXI)



(LXXII)



(LXXIII)



(LXXIV)

BROMINATION

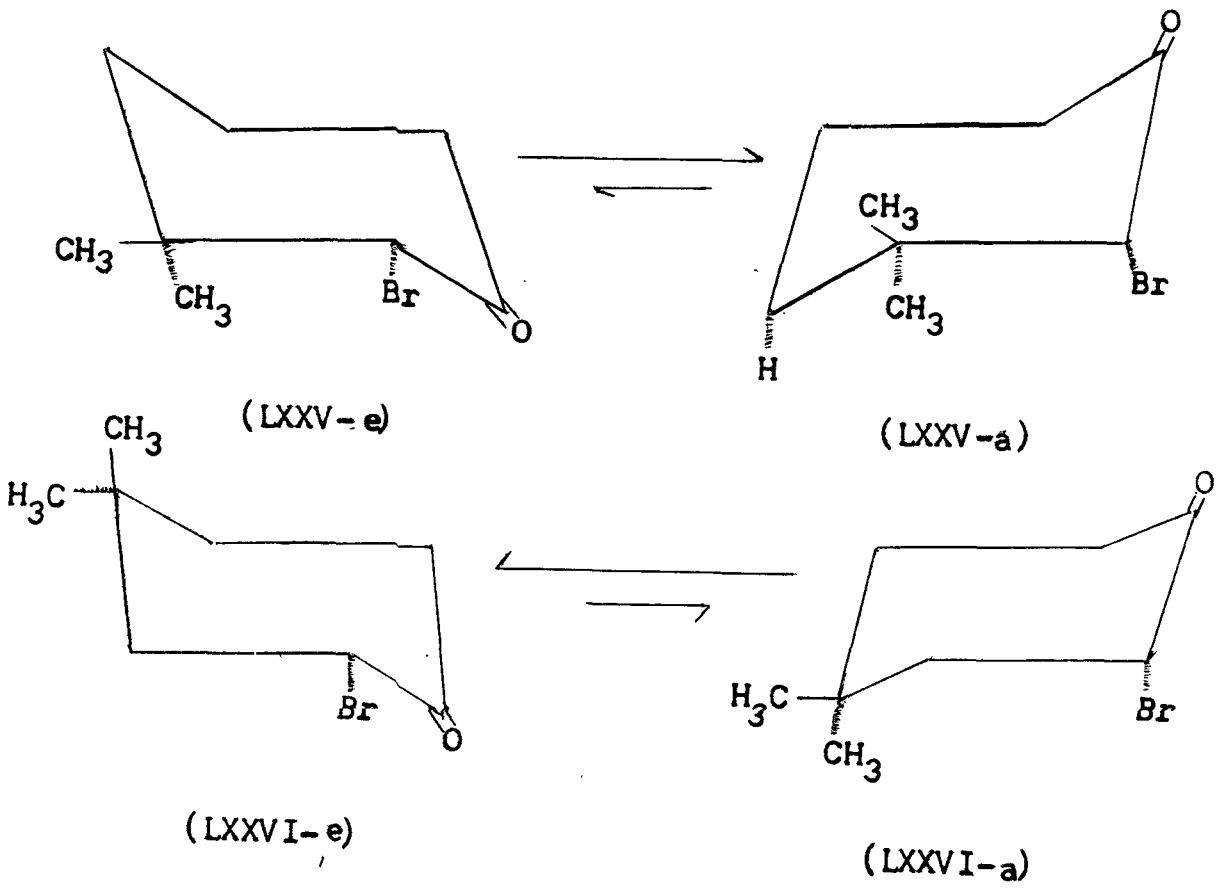
The ketones are brominated ordinarily in the presence of added or generated hydrogen bromide resulting, thereby, the formation of thermodynamically more stable product. In case of kinetically controlled bromination, the reaction may be effected by carrying out in the presence of an agent capable of removing hydrogen bromide as it is formed e.g. sodium acetate or by brominating the corresponding enol acetate in the presence of pyridine, sodium acetate or epichlorohydrin. The kinetic product may be the same[18] as the thermodynamic one or different[19-21] from it depending on steric factors.

α-Bromination of methylene and methyl ketones in the presence of base can not be stopped at the monobromoketone stage. The polybromoketones, thus, formed are cleaved under the basic conditions to form haloform and carboxylic acid.

The study of α-bromocyclanones is advantageous for two causes, first these compounds contain two strong dipoles, in close proximity, which are mutually repulsive to the greatest extent when steric strains are at a minimum and second, the approximate relative orientations of the two dipoles can be determined with the help of infrared spectroscopy. It was Corey[22] who advanced the theoretical arguments

led to the conclusion that α-bromocyclohexanone should exist almost completely at room temperature occupied axial position.

It was observed, with the help of infrared spectroscopy[22], that in methylated-2-bromocyclohexanones, the orientation of bromine in the stable conformation is sometimes axial and sometimes equatorial. Hence, in case of 2-bromo-3,3-dimethylcyclohexanone the more stable conformation is (LXXV-a, axial) and in the 4,4-dimethyl isomer, it is (LXXVI-e, equatorial). In (LXXV-e) the electric repulsion between C=O and C-Br dipoles destabilizes it to the extent of



some 2.7 K. cal/mole, while steric interactions between 1:3 H:Br in (LXXV-a) is found to destablise it by only about 0.4 K. cal/mole. So (LXXV-a) predominates over (LXXV-e). In case of the 2-bromo-4,4-dimethylcyclohexanone, however, steric repulsion between axial bromine and axial methyl in (LXXVI-a) out weighs the electrical effect in (LXXVI-e) and Br-equatorial is more stable. In case of steroidal bromo-ketones, these have achieved the conformation of maximum stability and the ring can not flip from one chair from to other. Although, a few bromoketones are known both in liable and stable forms, usually recognisable from the observation that under catalysed by HBr, the former can be isomerised to the later and the equilibrium is achieved by the enol forms.

Corey[23] characterised the bromination of steroidal ketones via the corresponding enols in several cases, and perhaps, generally by an effect which directs the incoming bromine substituent to the axial rather than the equatorial position. This is an indication that the axial epimer is formed faster rather than the equatorial one, opposing this effect is the classical steric effect which directs a large substituent such as (Br) to the less crowded equatorial orientation. The net result of these two effects which influence the relative rates of formation of the epimers with axial and equatorial bromine is clear in those cases where the bromoketone which is isolated as the unstable epimer,

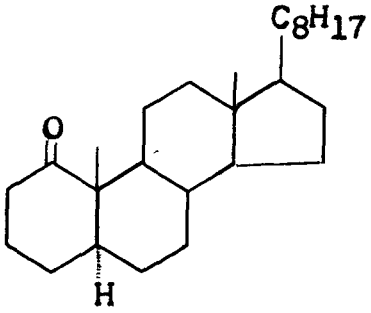
formed for kinetic rather than for steady state reasons. In such instances the importance of the non-steric effect is apparent since the major product has invariably been found to be the epimer with axial bromine.

Corey[22] has also developed a rule for predicting the orientation of bromine in all α-bromoketosteroids with ketones function in ring A, B or C and A/B cis or trans. One method applies to the stereochemistry of thermodynamically controlled bromination products and the other method applies to α-bromoketosteroids whose stereochemistry is kinetically controlled. In every case there is agreement between predicted and determined configuration at C (Br). In a number of cases it has been reported that the redetermination has been led by the prediction (and eventually reassignment of configuration). It has been shown, with exception, that the bromination of 5α,6β-dibromocholestan-3-one produced the 4α-derivative faster than the 4β-derivative although the latter is more stable. Similarly, the bromination of 3α-acetoxy-5α-cholestan-6-one afforded, as predicted, the 5α-bromo derivative which is isomerised to the 7α-bromo derivative by hydrogen bromide.

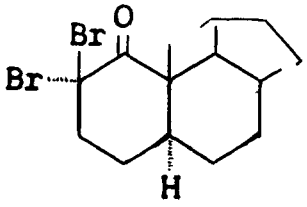
Corey[23] generalised the prediction for the stereochemistry to the kinetically controlled brominated products and the prediction is as 'the epimer which is formed faster

in the bromination of ketosteroids is that in which bromine is axial'.

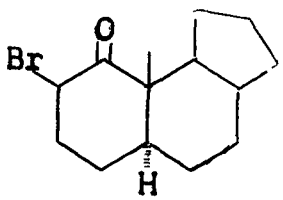
Sigg et al.[24] carried out the bromination of 5α-cholestan-1-one (LXXVII) with bromine in acetic acid in the presence of HBr (as catalyst) at room temperature and obtained three compounds, 2β-bromo (LXXIX), 2α,2β-dibromo (LXXVIII) and 2α-bromo ketones (LXXX).



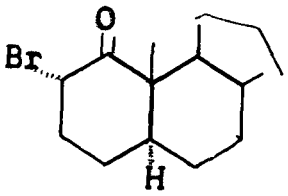
(LXXVII)



(LXXVIII)

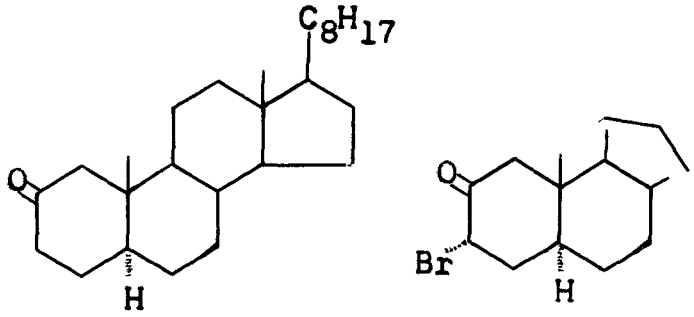


(LXXIX)



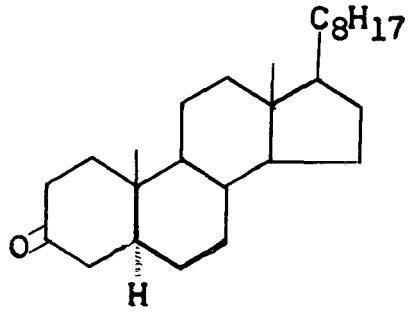
(LXXX)

3α-Bromo-5α-cholestan-2-one (LXXXII) was obtained from 5α-cholestan-2-one (LXXXI) by the treatment of bromine in acetic acid containing a trace of HBr.

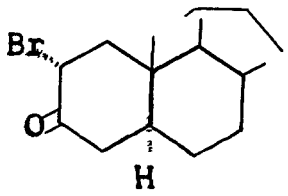


(LXXXI) (LXXXII)

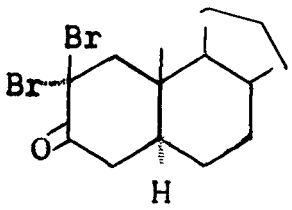
5α-Cholestan-3-one (LXXXIII) yielded different compounds under different conditions of bromination. When (LXXXIII) was reacted with Br_2/HBr in acetic acid for 10 minutes at room temperature, the product obtained was 2α-bromo-5α-cholestan-3-one (LXXXIV)[25,26]. Dibromination of the (LXXXIII) with bromine in acetic acid produced 2α,2β-dibromo-5α-cholestan-3-one (LXXXV)[27]. When bromination of the same compound (LXXXIII) was carried under similar conditions, i.e. bromine and acetic acid at room temperature, for 20 hours, yielded 2α,4α-dibromo-5α-cholestan-3-one (LXXXVI)[28].



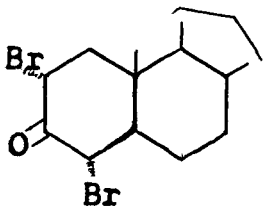
(LXXXIII)



(LXXXIV)

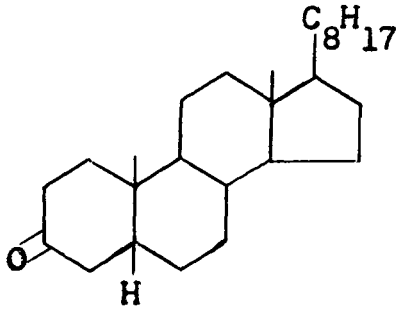


(LXXXV)

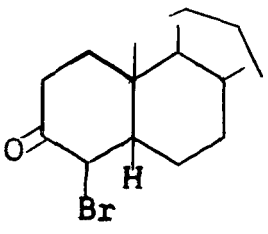


(LXXXVI)

Butenandt et al.[25] reported the bromination of 5β-cholest-3-one (LXXXVII) by Br_2/HBr in acetic acid at room temperature. The product obtained was 4β-bromo-5β-cholestan-3-one (LXXXVIII).

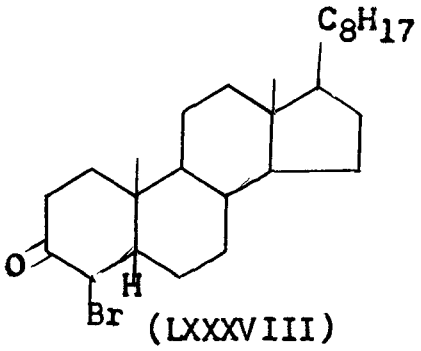


(LXXXVII)

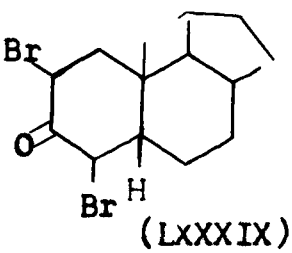


(LXXXVIII)

Bromination of (LXXXVII) was studied in detail by Shoppee and co-workers[29]. Kinetically controlled[30] monobromination of (LXXXVII) failed to yield the axial 4α-bromoketone and gave equatorial 4β-bromoketone (LXXXVIII). Dibromination of (LXXXVII) furnished diequatorial 2β,4β-dibromoketone (LXXXIX). Acid catalysed monobromination of (LXXXVIII) also afforded the 2β,4β-dibromoketone (LXXXIX).

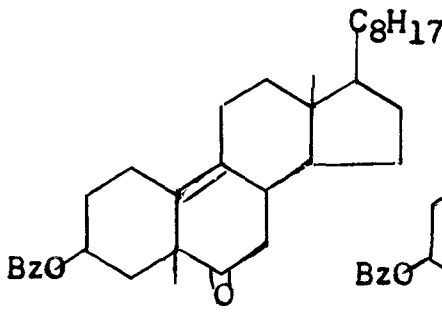


(LXXXVIII)

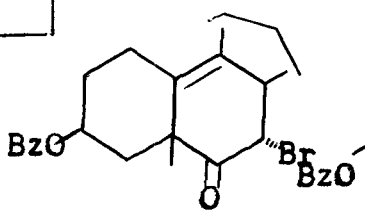


(LXXXIX)

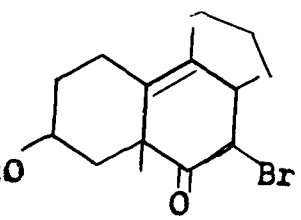
Bromination of 6-ketone (XC) yielded two products (XCI) major and (XCII) minor. The configuration of the bromine in (XCI) and (XCII) was determined with the help of IR and CD spectra which indicated half chair conformation for B ring in these compounds[31].



(XC)

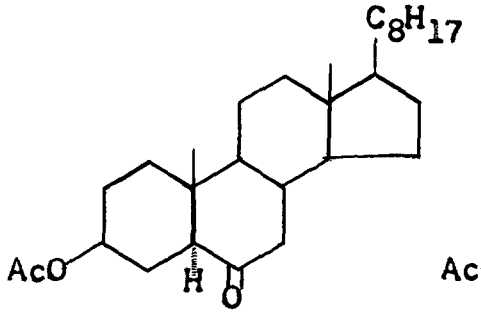


(XCI)

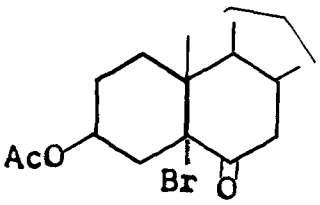


(XCII)

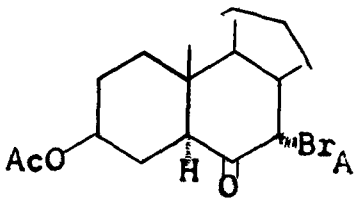
Bromination of 3β-acetoxy-5α-cholestan-6-one (XCIII) was carried out under different reaction conditions[23,32,33]. A variety of compounds were obtained by these reactions (XCIV-XCVII).



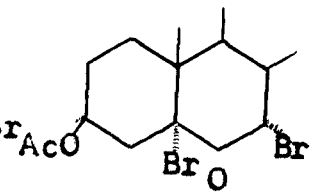
(XCIII)



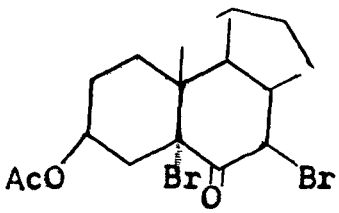
(XCIV)



(XCV)

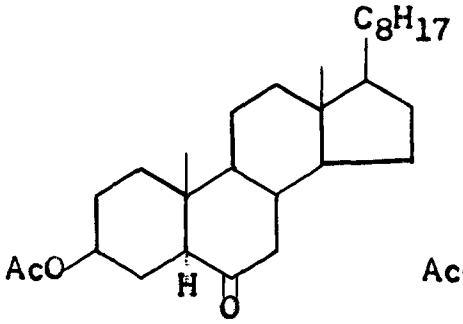


(XCVI)

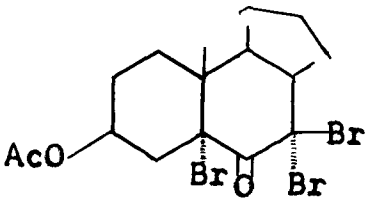


(XCVII)

When (XCIII) was treated with Br_2/HBr in ether-acetic acid, it invariably afforded 3β-acetoxy-5,7,7-tribromo-5α-cholestan-6-one (XCVIII)[34].

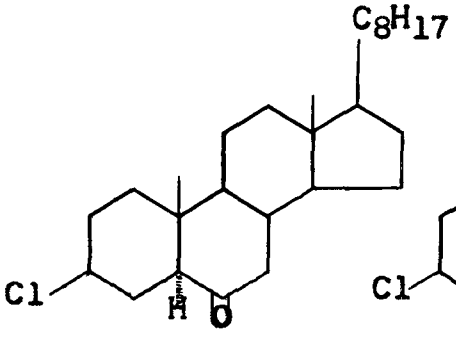


(XCIII)

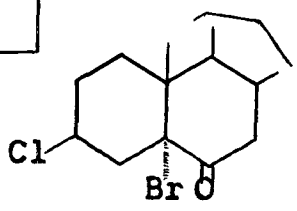


(XCVIII)

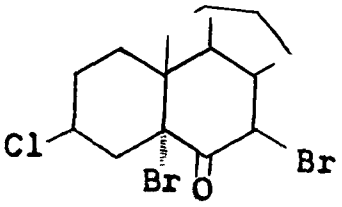
It has been reported[35] that the bromination of 3β-chloro-5α-cholestan-6-one (XCIX) under different reaction conditions, gave 3β-chloro-5α-bromocholestan-6-one (C) and when the reaction was carried out at room temperature or under reflux, the dibromocompound (CI) was obtained.



(XCIX)



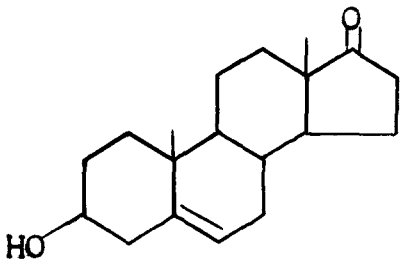
(C)



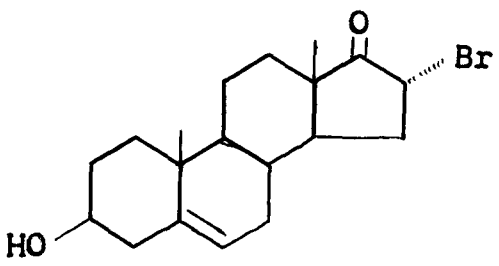
(CI)

Ellis[36] carried out bromination of steroidal ketones having an isolated double bond without any effect upon the double bond, 3β-hydroxyandrost-5-en-17-one (CII) gave

3β-hydroxy-16α-bromoandrost-5-en-17-one (CIII) on reaction with cupric bromide.

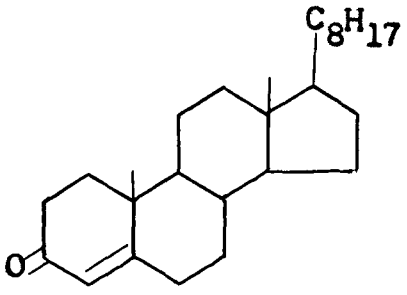


(CII)

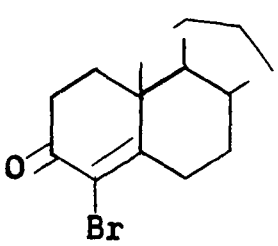


(CIII)

Kirk et al.[37] reported the bromination of cholest-4-en-3-one (LXVIII). They carried out the bromination in the presence of a proton acceptor (collidine) and 4-bromocholest-4-en-3-one (CIV) was obtained. Though, it was previously observed that 3-oxo- Δ^4-steroids on bromination with bromine in acetic acid or with N-bromosuccinimide in a suitable solvent, led, generally to allylic bromination with formation of the corresponding 6-bromo and 2,6-dibromo-3-oxo- Δ^4-steroids[38].

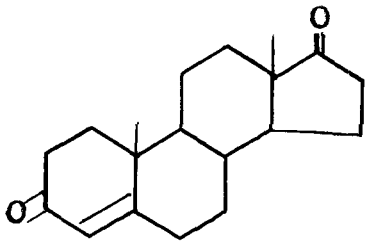


(LXVIII)

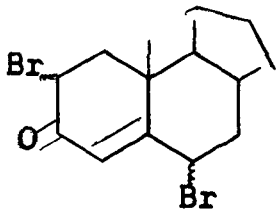


(CIV)

Djerassi and co-workers[38] dibrominated Δ^4-androstene-3, 17-dione (CV) with bromine and hydrogen bromide in acetic acid and got the dibromocompound (CVI).

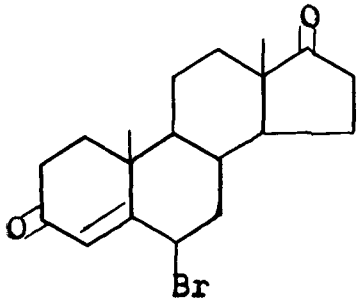


(CV)



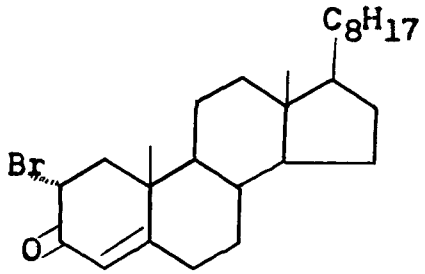
(CVI)

When (CV) was treated with equimolar quantity of N-bromosuccinimide in carbon tetrachloride, simply allylic bromination was noticed and 6-bromo- Δ^4-androstene-3,17-dione (CVII) was obtained[38]. 2α-Bromocholest-4-en-3-one (CVIII) on bromination with N-bromosuccinimide afforded

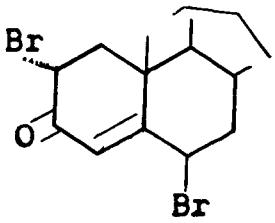


(CVII)

2α,6β-dibromocholest-4-en-3-one (CIX).

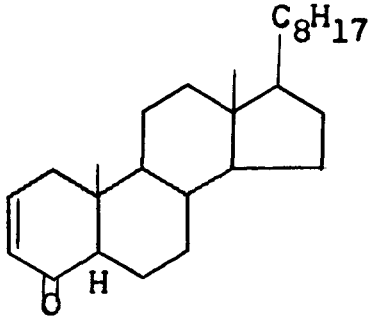


(CVIII)

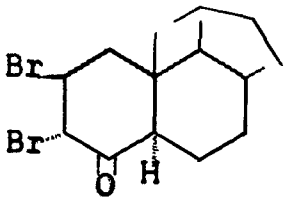


(CIX)

5α-Cholest-2-en-4-one (CX) in chloroform was treated with bromine at 20°, which provided 2β,3α-dibromo-5α-cholestan-4-one (CXI) i.e. the diaxial addition product[39].

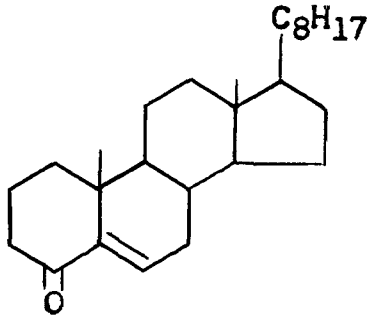


(CX)

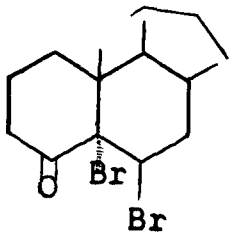


(CXI)

Bromination of cholest-5-en-4-one (CXII) afforded 5,6β-dibromo-5α-cholestan-4-one (CXIII)[39].

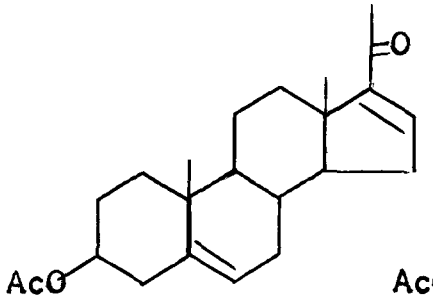


(CXII)

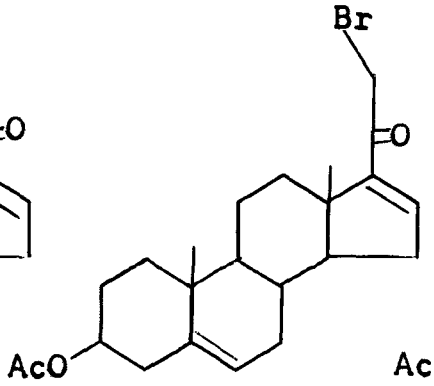


(CXIII)

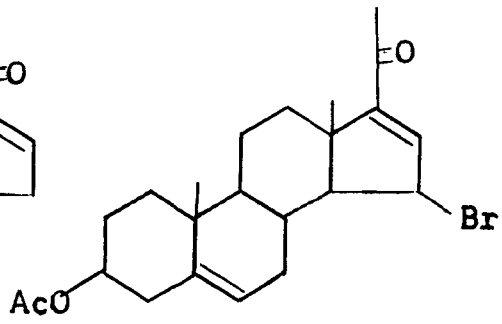
Reaction of 3β-acetoxy-5,16-dien-20-one (CXIV)[36] with cupric bromide in tetrahydrofuran was carried out with the thought of producing results and analogous to those obtained by Sollman and Dodson[40], i.e. bromine should be introduced at C_{15} to yield (CXVI) but the product obtained was 21-bromo derivative (CXV).



(CXIV)



(CXV)



(CXVI)

D I S C U S S I O N

Synthesis of Steroidal Ketones, Bromination and Biological Activities :

In the present work we have described the synthesis of steroidal ketones such as 5α-cholestane-3,6-dione (LXXIV) and cholest-4-en-3,6-dione (LXIV) and bromination of (LXIV) and its biological activities.

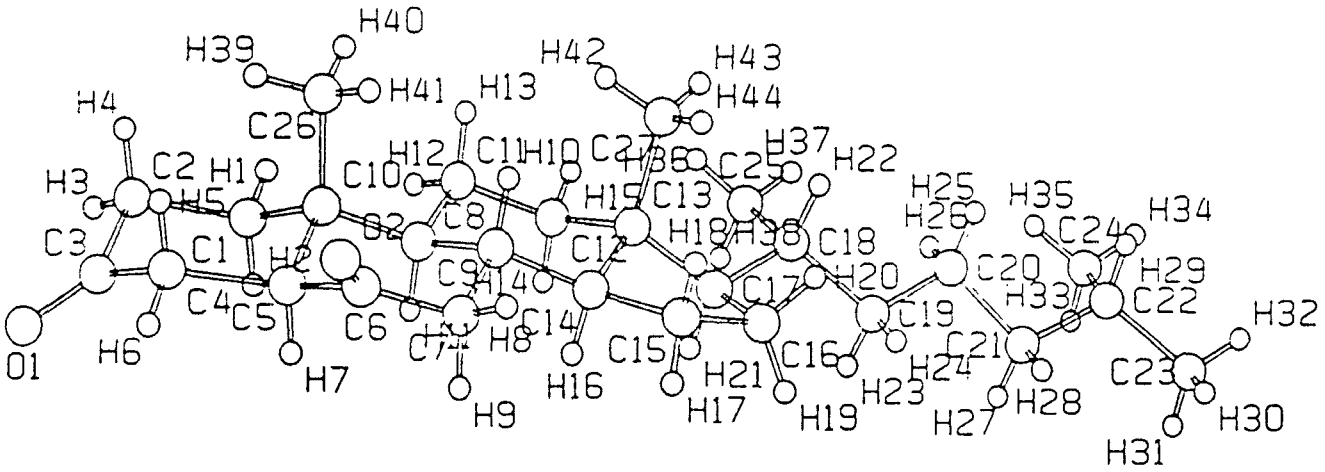
Heating under reflux of 2Σ,5,6β-tribromo-5α-cholestan-3-one (CXIX) with ethanol :

The tribromoketone (CXIX) was heated under reflux for 3 hours on the water bath in ethanol, then worked up in the usual manner to provide the brown solid which was chromatographed over silica gel affording a compound, m.p.170°C.

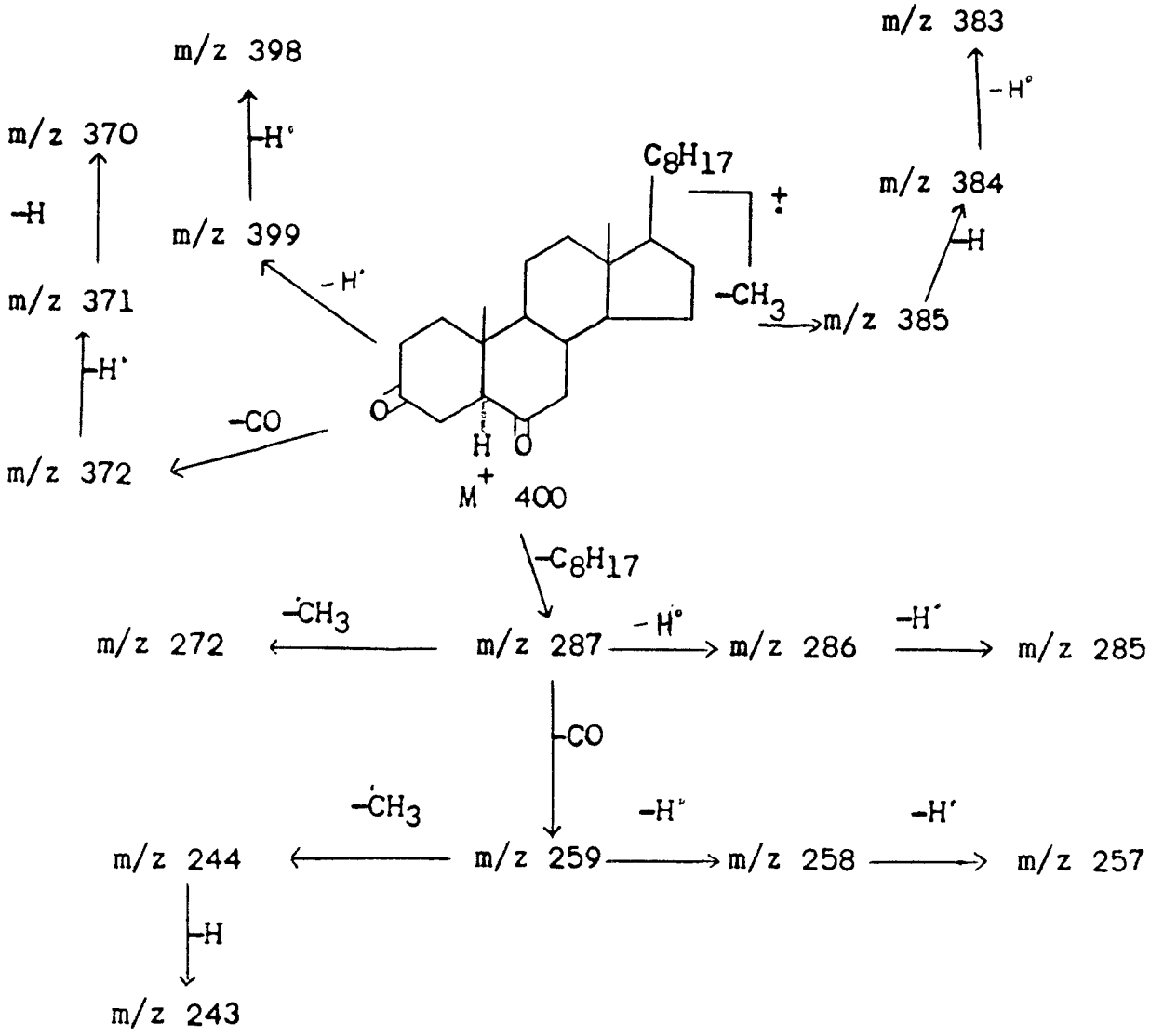
Characterisation of the compound having m.p. 170°C as 5α-Cholestane-3,6-dione (LXXIV) :

The compound with m.p. 170°C was correctly analysed for $C_{27}H_{44}O_2$. The IR spectrum of the compound showed bands at 1720 cm^{-1} (-C=O). Its ^{1}H-NMR spectrum exhibited its methyl protons at δ 0.95 (C_{10}-CH_3), 0.69 (C_{13}-CH_3), 0.92, 0.87 and 0.85 (other methyl protons). The ^{13}C-NMR showed two distinct

peaks at 211.26 and 209.112 for two carbonyl groups (C_6=O and C_3=O respectively). In the mass spectrum of the compound, the molecular ion peak was observed at m/z 400($M^{+\cdot}$). The other important fragment ion peaks were at m/z 399, 386, 373, 372, 288, 273 and 260. The formation of these fragment ions are explained in Scheme-1. The X-ray structure of the compound is also given in support of the structure. Thus on the basis of these analytical and spectral data the compound, m.p. 170°C was identified as 5α-cholestane-3,6-dione.



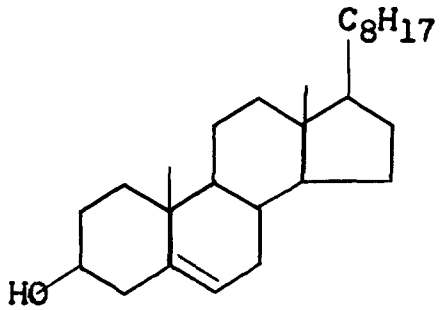
X-RAY STRUCTURE


m/z 383
-H°
m/z 398
m/z 370
H°
C8H17
m/z 384
-H
m/z 399
H
-H'
m/z 371
-CH3
m/z 385
H'
-CO
m/z 372
M+ 400
-C8H17
-CH3
-H°
-H'
m/z 272
m/z 287
m/z 286
m/z 285
CO
-CH3
-H'
-H'
m/z 244
m/z 259
m/z 258
m/z 257
H
m/z 243

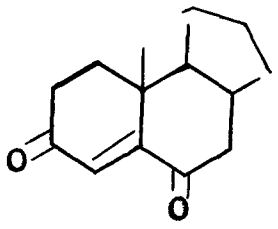
SCHEME-1

Reaction of 3β-hydroxycholest-5-ene with pyridinium dichromate (PDC) :

To a solution of 3β-hydroxycholest-5-ene in N,N-dimethyl formamide, the PDC was added at room temperature and stirred with the help of magnetic stirrer for 4 hours. After 4 hours the reaction mixture was poured onto the water and then worked up with ether and washed with water for several times so as to remove the chromate and DMF. The ethereal layer was dried over anhydrous sodium sulphate and the resulting solid was crystallised from methanol to afford the compound, m.p. 124°C.



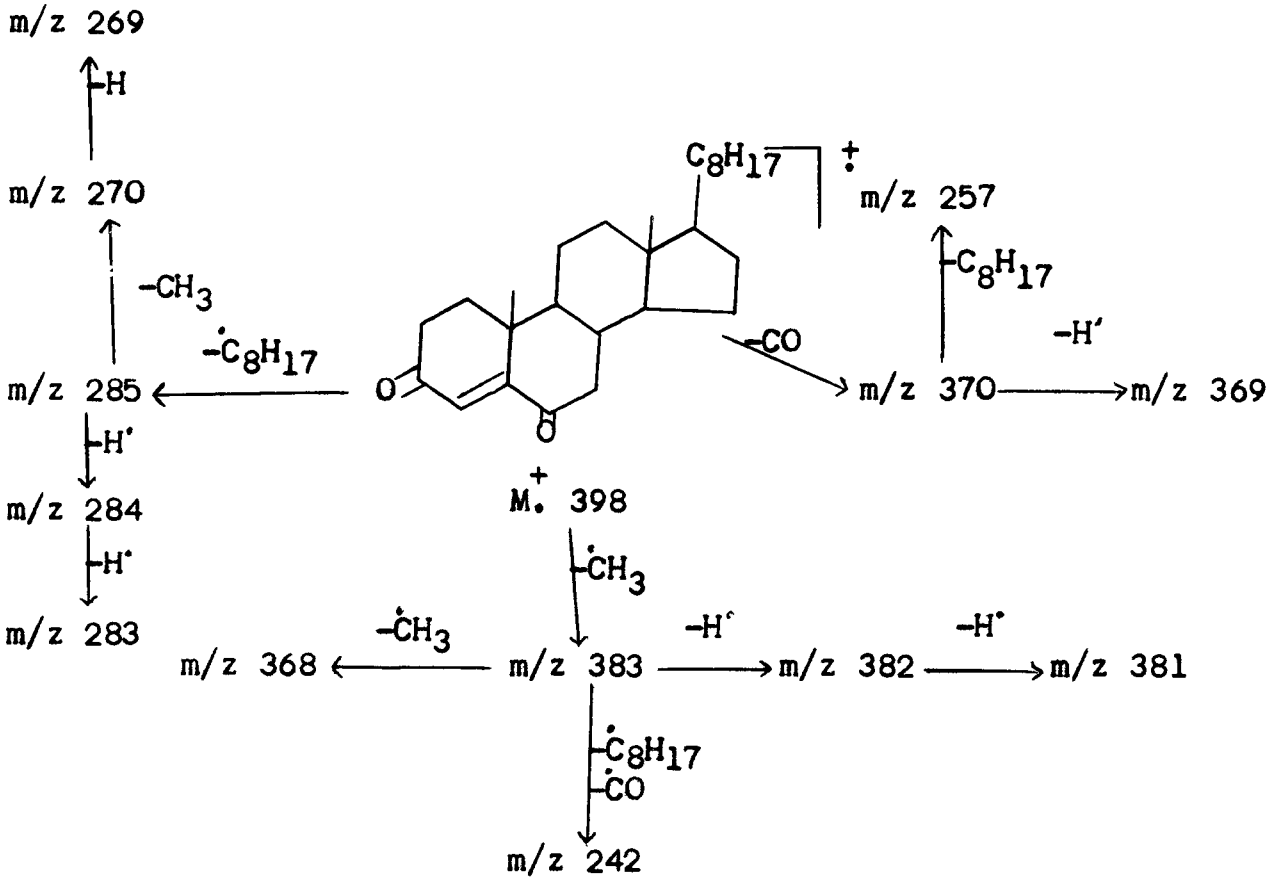
(LVIII)



(LXIV)

Characterisation of the compound (LXIV) having m.p. 124°C as Cholest-4-en-3,6-dione :

The compound, m.p. 124°C was correctly analysed for $C_{27}H_{42}O_2$. The U.V. spectrum showed λ_{max} at 250.5 and IR spectrum of the compound showed bands at 1700 and 1620 cm^{-1} (C=C-C=O). Its ^{1}H-NMR spectrum exhibited a singlet integrating for one proton at ∂ 6.15 and was assigned to the C_4-vinylic proton. The methyl protons appeared at 1.15 (C_{10}-CH_3) and 0.71 (C_{13}-CH_3), 0.91, 0.86 and 0.84 (for other methyl protons). The ^{13}C-NMR revealed two distinct peaks for (-C=C-) at 161.052 (C_5) and 125.404 (C_4). The mass spectrum of the compound gave molecular ion and some important fragment ions at m/z 398 ($M^{+}\cdot$), 397, 396, 383, 382, 381, 370, 369, 368, 285, 284, 283, 270, 269 and 257. The formation of these fragment ions were explained in the Scheme-2. On the basis of these values, the compound, m.p. 124°C was identified as cholest-4-en-3,6-dione (LXIV).


m/z 269
-H
m/z 270
-CH3
-C8H17
m/z 285
-H'
m/z 284
-H'
m/z 283
C8H17
+
m/z 257
-C8H17
-H'
-CO
m/z 370
m/z 369
M.+ 398
-CH3
-CH3
m/z 368
-H'
m/z 383
m/z 382
-H'
m/z 381
-C8H17
-CO
m/z 242

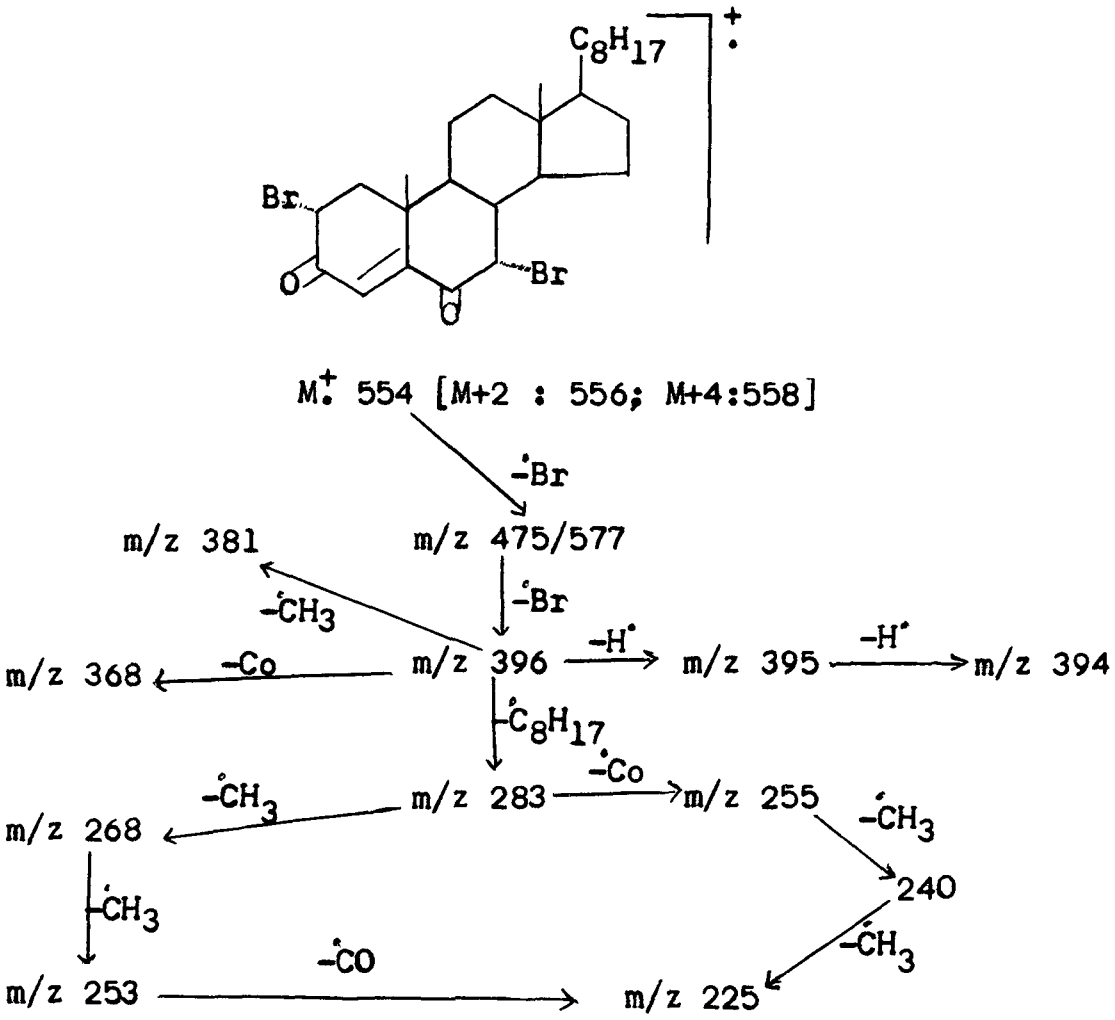
SCHEME-2

Reaction of cholest-4-en-3,6-dione (LXIV) with NBS (N-Bromosuccinimide) in presence of benzoyl peroxide (as catalyst) :

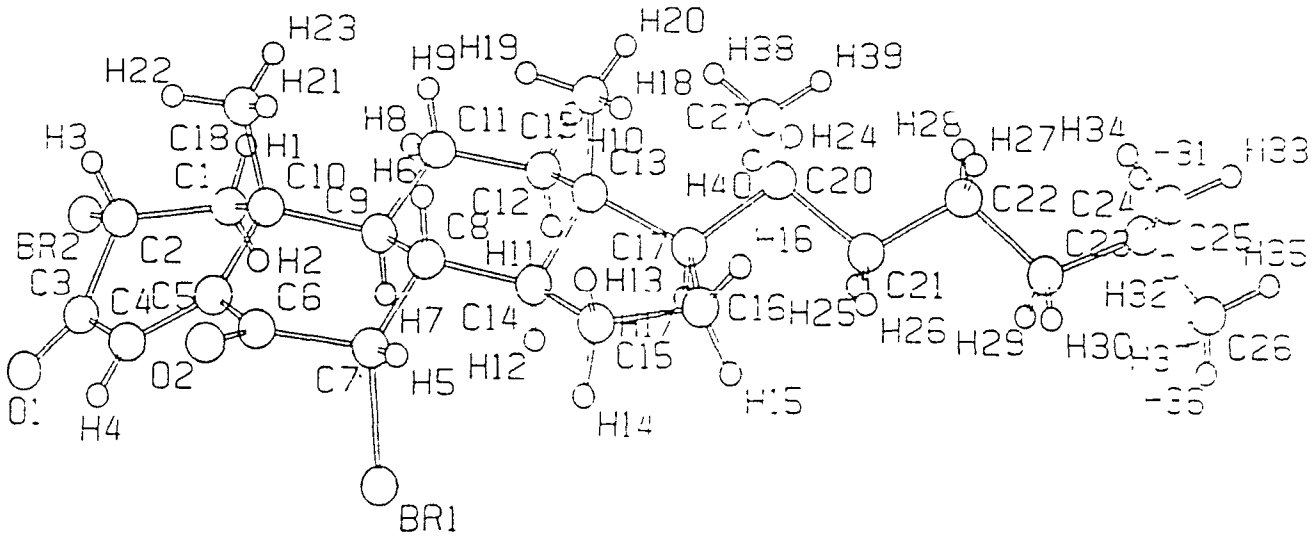
Cholest-4-en-3,6-dione was dissolved in benzene and a little (catalytic) amount of benzoyl peroxide is added then in the refluxed mixture, NBS (N-bromosuccinimide) was added in small portions over a period of 4 hours. After 4 hours, the solvent is evaporated under reduced pressure and the reaction mixture was taken in ether and washed with water and chromatographed over silica gel which afforded a compound crystallisable in light petrol having m.p. 181°C.

Characterisation of the compound having m.p. 181°C as 2α,7α-dibromocholest-4-en-3,6-diene (CXVI) :

The compound having m.p. 181°C was correctly analysed for $C_{27}H_{40}O_2Br_2$. The U.V. spectrum of the compound showed a λ_{max} at 240.8 and in IR spectrum, bands at 1700, 1620 (C=C-C=O) and 780 cm^{-1} (C-Br) were observed. Its ^{1}H-NMR exhibited a singlet at δ 6.32 integrating for one proton (C-4, vinylic). A double of doublet was observed at 4.85 for C_2 proton while a doublet at 4.38 for C_7 proton. The methyl protons appeared at 1.24 (C_{10}-CH_3), 0.74 (C_{13}-CH_3), 0.92, 0.87 and 0.85 for other methyl protons. Its ^{13}C-NMR showed peaks at 193.994 for C=O at C_6, 190.377 for C=O at C_3, 159.312 for C_5, 126.544 for C_4, 57.627 and 55.645 for C_7 and C_2 respectively. In its mass spectrum the molecular ion peak and some important fragment ions were found at m/z 554 ($M^{+\cdot}$), 556 (M+2), 558 (M+4) (these were in 1:2:1 ratio), 477/475, 476/474, 396, 395, 394, 381, 368, 283, 268, 255, 253 and 225. The fragmentation for these ions were explained in Scheme-3. On the basis of these analytical and spectral data the compound m.p. 181°C is characterized as 2α,7α-dibromocholest-4-en-3,6-dione (CXVI). Further support of this comes from its X-ray analysis.


C8H17
+·
Br
O
Br
O
M+· 554 [M+2 : 556; M+4:558]
-·Br
m/z 381
m/z 475/577
-·CH3
-·Br
m/z 368
-Co
m/z 396
-H·
m/z 395
-H·
m/z 394
-·C8H17
-·CH3
m/z 283
-·Co
m/z 255
m/z 268
-·CH3
240
-·CH3
-·CH3
-·CO
m/z 253
m/z 225

SCHEME-3



X-RAY STRUCTURE

Biological activities of 2α,7α-dibromocholest-4-en-3,6-dione (CXVI) :

2α,7α-Dibromocholest-4-en-3,6-dione (CXVI) on blind screening showed reduction in Palpebral aperture and frequency of spontaneous movements and that its higher doses affect the coordination of movement indicates that it possesses central depressant action. The decrease in body temperature could be due to specific central actions or be a part of generalised central depressant. The reduction in the tone of the limbs and abdomen could be due to the effect of motor neurons or due to generalised central depressant. The fact that the compound does not

increase the reaction time to both the mechanical and thermal nociceptive stimulii indicates that the compound probably does not have analgesic action. The lower doses i.e. 10 mg/kg and 20 mg/kg produce a narrowing the palpebral aperture but do not effect the movements indicate that they have only very mild central depressant actions if at all. The doses of 30 mg/kg and the higher doses reduce the movements, therefore, in the light of present study it can be seen that the dose of 30 mg/kg is the minimum dose that possesses clear cut central depressant actions. The bromoketone produced about 16% mortality at the dose of 50 mg/kg and 25% at 100 mg/kg.

The test compound i.e. 2α,7α-dibromocholest-4-en-3,6-dione (CXVI) is seen to possess unambigous central depressant activity and possibly a specific myorelaxant and hypothermic activity but no analgesic activity.

E X P E R I M E N T A L

3β-Hydroxy-5,6β-dibromo-5α-cholestane (CXVII) :

To a solution of cholesterol (14 g) in ether (100 ml) was added gradually Br_2 solution (5 ml Br_2 dissolved in 100 ml glacial acetic acid containing 2 gm of anhydrous sodium acetate) with stirring. The solution truned yellow and promptly set to a stiff paste of dibromide. The mixture was cooled and stirred with glass rod for 5 minutes to ensure complete crystallisation. The product was then filtered under suction and washed with cold ether-acetic acid (3:7) mixture until the filterate was completely colourless. The white dibromide is air dried (15 g), m.p. 113°C (reported[41], m.p. 112-113°C).

5,6β-Dibromo-5α-cholestan-3-one (CXVIII) :

3β-Hydroxy-5,6β-dibromo-5α-cholestane (10 g) was suspended in acetone (300 ml) in a three nacked flask fitted with a stirrer and a dropping funnel. The suspension was stirred for 5 minutes and Jones reagent (15 ml) was then added dropwised from a dropping funnel in the course of 15 minutes. The temperature of the reaction mixture during oxidation was maintained between 0-5°C by external cooling.

After complete addition, stirring was continued for further 15 minutes and cold water was then added (200 ml). The product thus obtained was filtered and washed with water thoroughly under suction and air dried to give the dibromo-ketone 8.5 (g), m.p. 74°C (reported[41], m.p. 73-75°C).

2Σ,5,6β-Tribromo-5α-cholest-3-one (CXIX) :

2.2 g of 5,6β-Dibromo-5α-cholestan-3-one was added in 200 ml of glacial acetic acid and 2 drops of HBr (as catalyst). The solution was stirred at room temperature with Br_2 (2.5 g, Br_2 in 33 ml acetic acid and few drops of HBr) for 1 hour. After addition of Br_2 solution, cold water was added and white solid was filtered under suction and air dried, 1.9 g, m.p. 101-103°C.

5α-Cholestane-3,6-dione (CXXIV) :

A solution of 2Σ,5,6β-5α-cholestan-3-one (1.6 g) in ethanol (150 ml) was refluxed for three hours. After three hours, the alcohol was evaporated under reduced pressure and solid mass was taken in ether. The ethereal solution was washed with water and then ether was removed and solid thus obtained was chromatographed over silica gel. The elution with petroleum ether (60-80°) and ether (2:1) provided a

solid which was crystallised from petroleum ether, 300 mg, m.p. 170°C (reported[42], 169°C).

Analysis found : C, 81.0; H, 11.0

Required : C, 80.9; H, 11.1%

I.R. : $\nu_{max.}$ 1720 cm^{-1} (C=O)

^{1}H-NMR ($CDCl_3$) : δ 0.95 (s, 3H, C_{10}-CH_3), 0.69 (s, C_{13}-CH_3), 0.92, 0.87 and 0.85 (for other methyl protons).

The ^{13}C-NMR showed two distinct peaks at 211.26 and 209.112 for two carbonyl groups (C_6=O and C_3=O respectively).

Mass : m/z 400(M^+), 399, 386, 373, 288, 273 and 260.

Preparation of PDC (Pyridiniumdichromate) :

Chromium trioxide (50 g) was dissolved in 50 ml of water and cooled, then 40.6 ml of pyridine is added. The resulting solution was cooled to -20°C by external cooling and then 200 ml of acetone is added and filtered immediately under suction, providing dark red shining crystals, m.p. 156°C.

Reaction of cholesterol with PDC : Cholest-4-en-3,6-dione (LXIV) :

To a solution of cholesterol (2 g) in 44 ml of N,N-dimethylformamide, 8.8 g of PDC was added and the reaction mixture was stirred at room temperature under anhydrous conditions for 4 hours. The reaction was monitored with the help of TLC plates. After 4 hours, 440 ml of water was added and the reaction mixture was worked up with ether in the usual manner. The ethereal solution was dried over anhydrous sodium sulphate. Evaporation of solvent provided yellow solid which was crystallized from methanol, 1.74 g, m.p. 124°C (reported[43], m.p. 122-123°C).

Analysis found : C, 81.4; H, 10.6

Required : C, 81.2; H, 10.8%

U.V. : $\lambda_{max.}$ 250.8 nm.

I.R.: ν_{max} 1700 and 1620 cm^{-1} (C=C-C=O).

^{1}H-NMR ($CDCl_3$) : ∂ 6.15 (s, 1H, C_4-vinylic), 1.15 (s, 3H, C_{10}-CH_3), and 0.71 (s, 3H, C_{13}-CH_3)
0.91, 0.86 and 0.84 (other methyl protons).

^{13}C-NMR : 161.052 and 125.404 for C_5 and C_4 respectively.

Mass : m/z 398 (M^{+}), 397, 396, 383, 381, 370, 369, 368, 285, 284, 283, 270, 269 and 257.

Reaction of cholest-4-en-3,6-dione (LXIV) with N-bromo-succinimide (NBS) in presence of benzoyl peroxide (as catalyst) : 2α,7α-Dibromocholest-4-en-3,6-dione (CXVI) :

A solution of 2 g of cholest-4-en-3,6-dione in 60 ml dry benzene and benzoyl peroxide (in catalytic amount) was added NBS (2 g) gradually in small portions for a period of 3 hours under reflux conditions. The solution was further refluxed for 1 hour. The solvent was evaporated under reduced pressure. The resulting solution was taken in ether, washed with water and sodium bicarbonate (5%) and again with water. The ether layer is collected and dried over anhydrous sodium sulphate. Removal of the solvent provided a brown solid which was crhomatographed over silica gel. Elution with petroleum ether and ether (8:1) provided the white solid which was crystallised from petroleum ether, 1.52 g, m.p. 181°C.

Analysis found : C, 58.02; H, 7.44

Required : C, 58.27; H, 7.19%.

U.V. : $\lambda_{max.}$ 240.8, mμ.

I.R.: ν_{max} 1700, 1620 (C=C-C=O) and 780 cm^{-1} (C-Br).

^{1}H-NMR ($CDCl_3$) : δ 6.32 (s, 1H, C-4 vinylic), 4.85 (dd, 1H, C-2), 4.38 (d, 1H, C-7), 1.24 (C_{10}-CH_3), 0.74 (s, 3H, C_{13}-CH_3) and 0.92, 0.87 and 0.85 (other methyl protons).

^{13}C-NMR : 193.994 (C_6=O), 190.377 (C_3=O), 159.312 and 126.544 (C_5=C_4), 57.627 and 55.645 for C_7 and C_2 respectively.

Mass : m/z 544 ($M^{+}_{\cdot}$), 556 (M+2), 558 (M+4) (in 1:2:1 ratio), 477/475, 476/474, 396, 395, 394, 381, 368, 283, 268, 255, 253, 240 and 225.

PHARMACOLOGICAL TESTING :

Gross Behaviour Test of 2α,7α-dibromocholest-4-en-3,6-dione (CXVI) :

The test was carried out by the method of Smith[44] with modification in the light of Irwin's method[45]. The method is a simple but comprehensive detecting a large number of neuropharmacological actions. As it is usual to begin with 30 mg/kg for blind screening because the test compound has

no pharmacological history[46]. However the lower as well as higher doses were also used. The compound was suspended in propylene glycol and injected in tetraperitoneally. The effect of bromoketone was studied at the dose of 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg, 50 mg/kg and 100 mg/kg. Each dose was tested in 6 mice of either sex weighing 20-25 gms. The animals were observed for various parameter listed in Table-I. The mechanical and thermal nociceptive stimulus was given by applying a sheathed bull dog clamp at the tail and placing the animal on Eddy's Hot Plate at 55.5°C respectively. The animals were continuously observed for 6 hours and then at 24 hours. They were observed subsequently for mortality at 24 hours for 6 days.

The bromoketone (CXVI) was found to produce narrowing of the palpebral aperture at all the doses. The spontaneous movements were found to be increasing by decreased at the dose of 30 mg/kg, 40 mg/kg, 50 mg/kg and 100 mg/kg. The compound did not affect the movements at 10 mg/kg and 20 mg/kg. The higher doses viz. 50 mg/kg and 100 mg/kg produced a staggering gait. All the doses increased the rate of respiration and decreased the depth of respiration. The body temperature was found to be reduced by all the doses of the compound except the dose of 10 mg/kg. Similarly the limb and abdominal tone was reduced by all the doses except

the dose of 10 mg/kg. The compound did not increase the reaction time to the mechanical as well as nociceptive stimulus. Straub's response was not observed. The reflexes were also not effected. Salivation, Lacrimation and excessive urination were also not seen. The result is presented in Table-I.

TABLE-I. Effective of various doses of the 2α,7α-Dibromo-cholest-4-en-3,6-dione on behaviour of mice

S. No.	Parameters	10 mg/ kg	20 mg/ kg	30 mg/ kg	40 mg/ kg	50 mg/ kg	100 mg/ kg
1.	Time of first unusual S/S	50 mints	50 mints	50 mints	50 mints	50 mints	50 mints
2.	Movements	N	N	↓	↓	↓↓	↓↓
3.	Co-ordination	N	N	↓	↓	↓	↓
4.	Palpebral aperture	↓	↓	↓	↓	↓	↓
5.	Alertness	N	N	N	↓	↓	↓
6.	Grooming	N	N	N	N	N	N
7.	Restlessness	A	A	A	A	A	A
8.	Stereotypy	A	A	A	A	A	A
9.	Tremour	A	A	A	A	A	A
10.	Convulsions	A	A	A	A	A	A
11.	Rate of respiration	N	↑	↑	↑	↑	↑
12.	Depth of respiration	N	↓	↓	↓	↓	↓

Contd...

TABLE-I Contd...

13.	Body Temperature	N	↓	↓	↓	↓	↓
14.	Piloerection	A	A	A	A	+ve	+ve
15.	Limb tone	N	N	↓	↓	↓	↓
16.	Abdominaltone	N	N	↓	↓	↓	↓
17.	Pinna reflex	N	N	N	N	N	N
18.	Corneal reflex	N	N	N	N	N	N
19.	Lacrimation	A	A	A	A	A	A
20.	Salivation	A	A	A	A	A	A
21.	Urination	N	N	N	N	N	N
22.	Excessive defecation	No	No	No	No	No	No
23.	Startle response	+ve	+ve	+ve	+ve	+ve	+ve
24.	Touch response	+ve	+ve	+ve	+ve	+ve	+ve
25.	Straub's response	-ve	-ve	-ve	-ve	-ve	+ve
26.	Mortality	Nil	Nil	Nil	Nil	1/6	1/4

N = normal, A = Absent, +ve = Positive, -ve = Negative

↑ =Increased, ↓ = Decreased.

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